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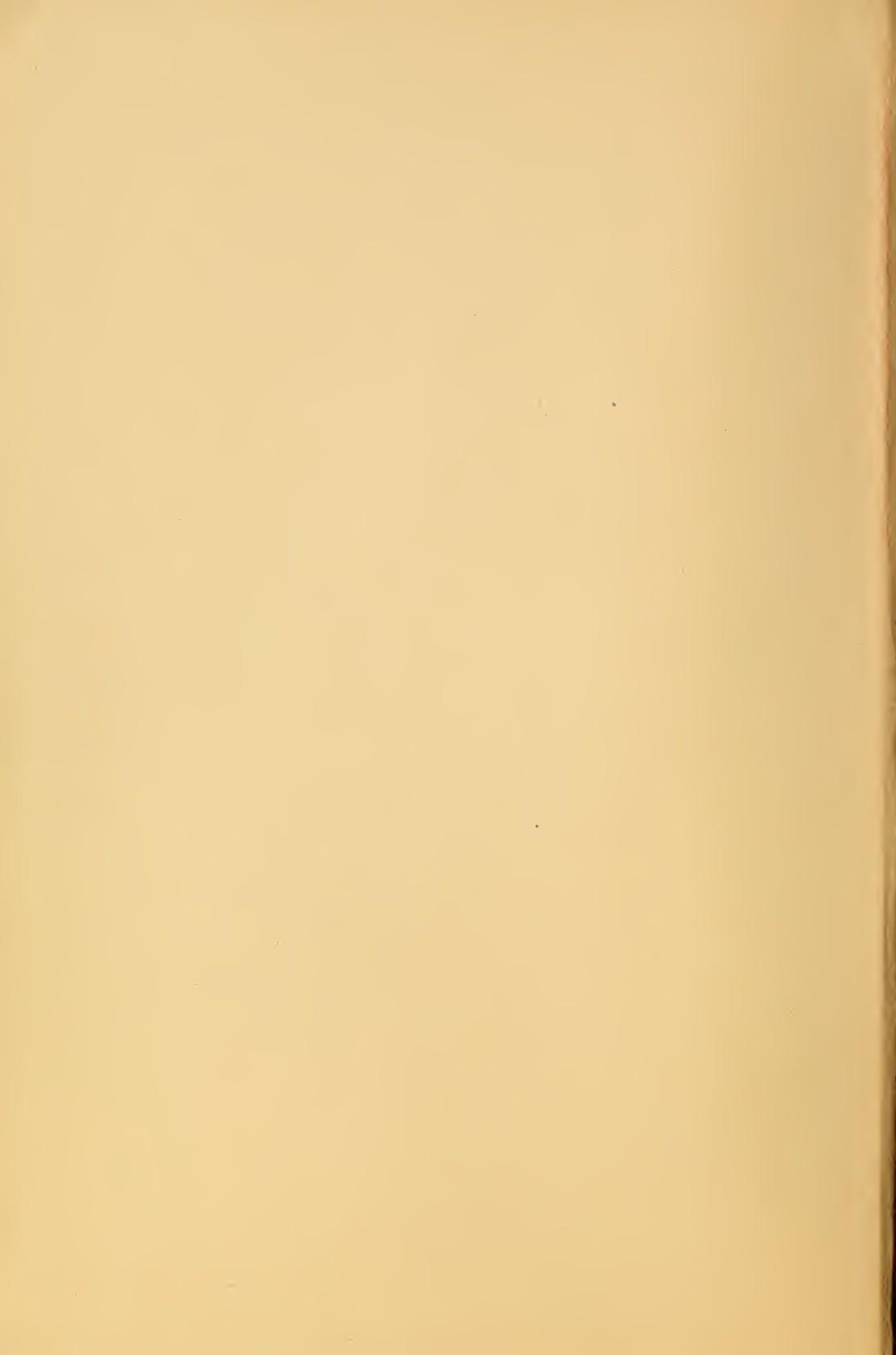
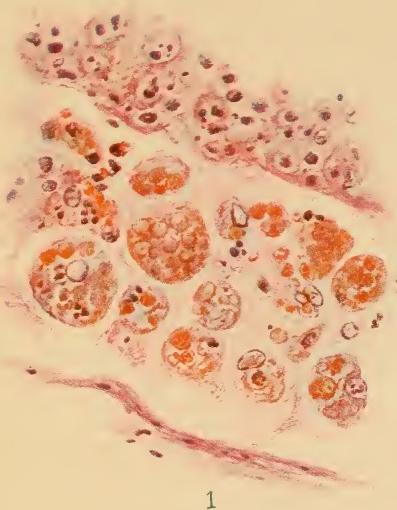


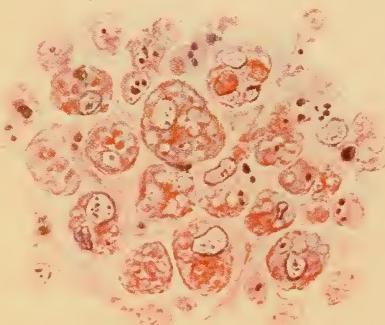
PLATE I



1



3



Inf. fibro

2



4

Lymph-nodes and liver of splenectomized dogs receiving haemolytic serum and showing phagocytic cells containing red blood-corpuscles.

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THE
SPLEEN AND ANÆMIA
EXPERIMENTAL AND CLINICAL STUDIES

RICHARD MULS PEARCE, M.D., Sc.D.

EXPLANATION OF PLATE I.

The drawings were made with the camera lucida and with a Spencer microscope, objective, 4 mm., ocular, 8.

Figs. 1, 2, and 3 represent lesions in a dog that was splenectomized on March 11, 1912, received hemolytic serum on March 14, and died on March 15. The actual lapse of time was about thirty-six hours.

FIG. 1. Peripheral sinus of a mesenteric lymph node containing large numbers of endothelial cells filled with red blood corpuscles and occasionally also polymorphonuclear leucocytes.

FIG. 2. Similar cells in a central sinus of the same lymph node.

FIG. 3. A section of liver with two Kupfer cells containing red blood corpuscles.

FIG. 4. Similar to figure 3, but from a dog that was splenectomized on July 19, 1911, received hemolytic serum on March 8, 1912, and died after forty-eight hours.

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EXPLANATION OF PLATE I.

THE SPLEEN AND ANÆMIA EXPERIMENTAL AND CLINICAL STUDIES

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16 ILLUSTRATIONS, COLOR AND BLACK AND WHITE.



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March 18th

TO THE LIBERAL AND FAR-SIGHTED BENEFACTOR
WHO ESTABLISHED, UNDER THE CLOAK OF ANO-
NYMITY, THE JOHN HERR MUSSER DEPARTMENT OF
RESEARCH MEDICINE IN THE UNIVERSITY OF PENN-
SYLVANIA, THIS BOOK IS GRATEFULLY DEDICATED



PREFACE

IN this volume splenectomy is considered, first, as a means of studying experimentally in animals the relation of the spleen to blood destruction and regeneration and, second, as a therapeutic procedure in the treatment of diseases of man accompanied by anæmia. No attempt is made to discuss injuries, infections and tumors of the spleen, or, except incidentally, the problems—leucocytosis and leucaemia—of the white blood-cells. The emphasis is on the side of the red blood-cell and the relation of the spleen to the quantitative and qualitative changes which the red cell may undergo.

The chapters on experimental and metabolic observations are based on some twenty odd studies carried out during the past five years in the John Herr Musser Department of Research Medicine of the University of Pennsylvania, and reported from time to time in medical periodicals, under the general title of “The Relation of the Spleen to Blood Destruction and Regeneration and to Hæmolytic Jaundice.” These various papers have been rearranged and largely rewritten and brought into relation with the general literature so as to offer a consecutive comprehensive presentation of the general experimental problem.

Such experimental studies are obviously of importance in connection with the diseases of man characterized by splenomegaly, with anæmia or jaundice, or both, and in connection with which splenectomy, as a therapeutic procedure, has recently been so widely tried. Clinical studies of the splenomegalies and of the results of splenectomy in man are therefore presented by Dr. Krumbhaar. These chapters bring into one volume for the first time the modern

views concerning the classification, diagnosis and treatment of the non-infectious splenomegalies characterized by blood destruction. The final section by Dr. Frazier gives details of the technic of the operation of splenectomy in man. This has been included, partly to complete our presentation, but chiefly to bring out new points concerning the operation which have been gained as the result of its widely extended use during the last few years.

Acknowledgments are due, and are gratefully made, to those assistants,—J. H. Austin, M.D.; Harry Dubin, Ph.D.; A. B. Eisenbrey, M.D.; Samuel Goldschmidt, Ph.D.; H. T. Karsner, M.D.; E. B. Krumbhaar, M.D.; J. H. Musser, Jr., M.D.; M. M. Peet, M.D., and O. H. Perry Pepper, M.D.,—who assisted in the experimental work and thus made possible the many detailed studies, and likewise to the Board of Scientific Directors of the Rockefeller Institute for Medical Research, who by grants of money from time to time furthered the distinctly laboratory studies. To the editors of the *Journal of Experimental Medicine*, *Archives of Internal Medicine*, *American Journal of the Medical Sciences*, *The Journal of the American Medical Association* and the *New York Medical Journal*, we are also indebted for the privilege of reproducing in the present form material originally appearing in their journals.

The material presented under the headings of experimental and metabolic studies constitutes the substance of the Cartwright Lectures delivered in New York on October 24 and 25, 1916, under the auspices of the Association of the Alumni of the College of Physicians and Surgeons of Columbia University.

September, 1917.

THE AUTHORS.

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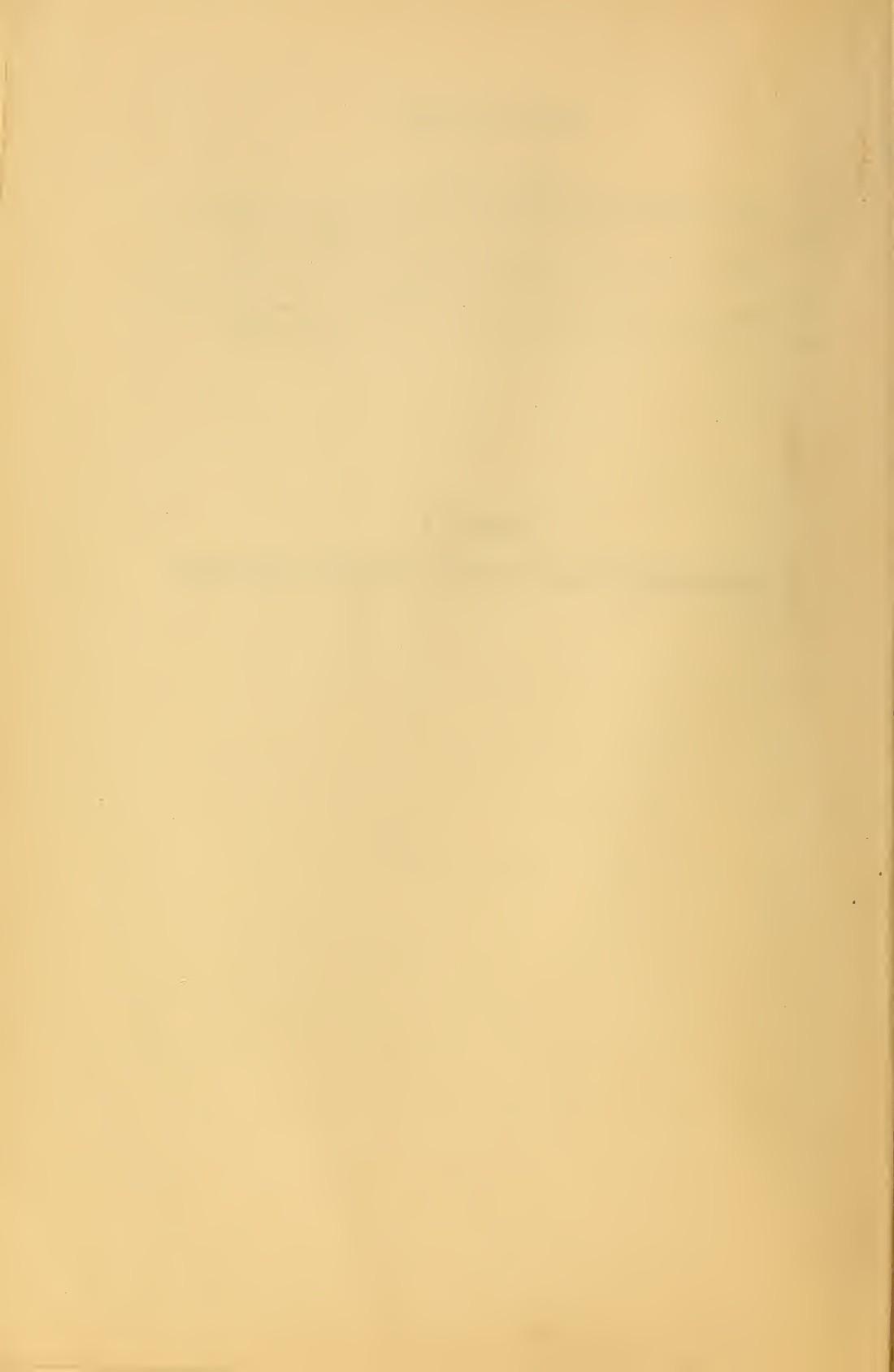
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PART I
EXPERIMENTAL STUDIES BY R. M. PEARCE



THE SPLEEN AND ANÆMIA

CHAPTER I

THE HISTORY OF EXTIRPATION OF THE SPLEEN

THE statement is frequently seen that the ancients practised removal of the spleen in the belief that it improved the wind of runners. It is also stated in the older literature that the swift giraffe is spleenless, an erroneous supposition that may have had something to do with the views of the ancients concerning the spleen and the speed of runners. Aristotle¹¹ assumed that the spleen is not necessary for the maintenance of existence, and Erasistratus insisted that it is of no use whatever. Galen more conservatively considered it an organ full of mystery (*Mysterii pleni organon*) and that it probably removed the melancholy of the blood going from the liver to the stomach. The first positive statement as to its extirpation (not excision) is made in Pliny's³⁵⁷ Natural History. An old English³⁵⁷ translation runs as follows: "This member (the spleen) hath a proprieite by it selfe sometimes, to hinder a man's running; whereupon professed runners in the race that bee troubled with the splene, have a devise to burne and waste it with a hot yron. And no marveile; for why? They say that the splene may be taken out of the body by way of incision, and yet the creature live neverthelesse; but if it be man or woman that is thus cut for the splene, he or she looseth their laughter by the means. For sure it is that intemperate laughers have always great splenes." In connection with the last state-

ment it is interesting that the modern German word for hypochondriasis is "Milzsucht." Pliny is said to have performed experiments involving the removal of the spleen from dogs, but this is probably merely an incorrect quotation of the foregoing. The belief that splenectomy improved the wind of runners was not lost sight of in the middle ages, as is shown by the couplet quoted by Brogsitter⁶⁰ from Murer's *Belägerung von Babylon*:

"Ich han mir lon dass milz schnyden,
Dass ich mag laufen wegt und veer."

He also quotes Paracelsus, who considered the spleen "des Leibers und Lebens Unkraut," and advised physicians to excise it whenever possible. Van Helmont, on the other hand, attributed to it important and even vital functions.

The first authentic experimental splenectomy in the dog was performed by Zambeccari⁴⁸² in 1680, with apparently an uneventful recovery. Marcello Malpighi,²⁷² the discoverer of the lymphoid follicles of the spleen, had previously described the effects of ligating the splenic vessels of a dog. It not only quickly recovered from the operation, with no noticeable injury to health, but became more voracious and much lazier and fatter. Nothing abnormal was noted in the stools. A second operation on this dog, performed some time later, showed almost complete disappearance of the spleen, but no other changes except slight enlargement of the liver and engorgement of the mesenteric vessels. Clarke⁷⁸ successfully extirpated a dog's spleen in 1676, and during the year following found no changes except that the animal became much fatter.

The celebrated pathologist, Morgagni,²⁹⁹ states that he

and Vallisnerius found during a period of five years' study no change in the size, disposition, or fertility of dogs whose spleens had been removed. J. H. Schultze³⁹⁸ early practised splenectomy on dogs with a view to the application of the operation to human beings. Harvey and his pupils are frequently quoted as having extirpated the spleen in dogs, but we have not been able to find such accounts in Harvey's works.

Observations on experimental splenectomy are more numerous in the nineteenth century. Assolant,²⁰ found that in dogs the blood became more watery, with the appearance of scurvy-like symptoms and fatal peptic ulcer. He states that Dupuytren lost almost half of forty dogs after splenectomy. Those that survived recovered in two or three weeks and acquired abnormal appetites. Spitta and Mayo found increase in weight, and Mayer increased tendency to sleep. Saunders reported no change in bile formation. A. S. Schultze³⁹⁹ removed the spleen of twenty-four animals (dogs, cats, goats, rabbits), losing only one puppy. He states that the operation is followed by lessened fertility, greater inclination and ability to run far, and at first a decreased secretion of bile. Czermak,⁸⁵ working with dogs, rabbits and cats, found that two-thirds of the animals survived, and showed lessened fertility and enlargement of the mesenteric lymph-nodes. He noted that the spleen became greatly congested after feeding. Vulpian,⁴⁵⁹ on the other hand, found no change in fertility. Enlargement of the lymph-nodes after splenectomy was noted by Tiedemann and Gmelin,⁴³⁴ Hyrtl,¹⁹² Mayer,²⁸⁰ Führer and Ludwig,¹³² Eberhard,¹⁰⁰ and Simon.⁴⁰⁹

Mayer maintained also that the extirpated spleen was

easily replaced by a newly-formed organ. This was confirmed by Eberhard, working on the frog, and by certain French and Italian investigators (Philippeaux,³⁵⁰ Eternod,¹⁰⁷ Tizzoni,⁴³⁷). More careful later work (Peyrani,³⁴⁸ Tizzoni,⁴³⁸ Ceresole,⁶⁹ Tedeschi,⁴³⁰), however, showed that when the spleen had been completely removed no regeneration took place; but, if a small portion was left *in situ*, it might hypertrophy and simulate complete regeneration (Philippeaux,³⁵¹ Laudenbach²³⁹). Even this, however, has been denied by Peyrani³⁴⁹ and Ceresole.⁶⁹ Bardeleben³⁴ found that extirpation of both spleen and thyroid was almost invariably fatal. Mosser³⁰² noted a stimulation of the bone-marrow.

Thus we find that before the year 1875 numerous experimenters, working on dogs, cats, goats, rats, mice, guinea-pigs, sheep, rabbits, frogs, and one (Eternod) on a fox, had found that the spleen was not necessary to life. Though one out of four splenectomized animals died (usually from peritonitis or pneumonia), the others quickly recovered and enjoyed good health. The most constant findings were increased appetite and eventual gain in weight. At autopsy, enlargement of the mesenteric lymph-nodes was frequently found, with occasionally enlargement of the liver, congestion of the splanchnic vessels, and, according to Mosser and Schindler,³⁰³ stimulation of the bone-marrow. The power to regenerate after extirpation was denied, though it was shown that if small amounts of splenic tissue are left behind, these possess great capacity for hypertrophy.

The first recorded splenectomy on a human being is the celebrated operation performed in Naples in 1549, by Zaccarelli, at the instance of Fioravanti,¹¹⁷ whose description follows:

In the month of April I was called to a Greek woman, the wife of a Greek centurion, or war captain, who lived at Panormus, near the Garden of Marinus de Terra Nova. Her name was Maruella, and she was twenty-four years old. Her spleen was stopped up (*oppilatus*) and grew to such a size that the body could not have held a larger one. She had been visited by several doctors and had been told that if she wished to be cured it would be necessary to take the spleen out of the body. The captain himself came to me and took me with him to visit his wife; she desired of me the removal of the spleen. For this purpose I invited an old man named Adrian Zaccarelli, from the town of Palum, in the kingdom of Naples, who was very skilled in surgery. With him I proceeded to the operation. The old man made an incision in the body and immediately the spleen protruded from the body. After we had separated it from the membranes we pulled it entirely out and sewed the body up, leaving only a little hole (*spiraculo exiguo relicto*). This I cured with *oleum hypericonis*, incense powder, mastix, and so on. In this manner she was cured in twenty-four days. When taken out of the body the spleen weighed thirty-two ounces.

It should be said that some writers have doubted the veracity of this description, and Simon has suggested that on account of the discrepancy in the size of the tumor before and after removal it may have been an ovarian cyst.

Two other equally doubtful reports of successful splenectomy in the sixteenth century are at hand. Baillon²⁵ tells in a few words how, in 1578, an unknown operator removed the spleen,

“qui secuit prius superiore parte ligata; convaluit æger. Este igitur splentam necessarius?” (Which he cut after the upper portion had been tied; the sick man recovered. Is then the spleen so necessary to life?)

Rousset³⁸⁸ also describes the successful removal, by a certain Doctor Viard, of a spleen which had already protruded through a wound in the left side.

In the seventeenth century two cases of total removal

of the spleen are recorded. Timothy Clarke's⁷⁸ case was reported by an eye-witness, Dr. Dovbeny Turbevile. A certain William Panier, of Somerset, in an attempted suicide drove his butcher's knife into his left side. The spleen, part of the omentum, and the intestines protruded from the wound, and his companions left him for dead. Three days later a surgeon replaced the intestines, cut away the spleen and omentum, and sewed up the wound. The patient quickly recovered, was quite well a year later, and then migrated to New England, where he lived happily and in good health for some years. The second case was of like character. Nicolaus Matthia,²⁷⁹ the town surgeon of Colberg, in 1678, was sent by the magistrate to a neighboring town to see a young man who had been injured by a knife-thrust in the left side of the abdomen. The protruding spleen was pulled entirely outside the body and ligated. Three days later the spleen was removed and the bleeding controlled with styptics. The patient recovered completely in three weeks, and six years later was in good health.

Thus physicians began to realize that the spleen was not necessary for life. However, in spite of several similar successful splenectomies in the eighteenth century (Gerbezius, 1700;¹¹² Ferrerius, 1711;¹¹² South-Wilson, 1743⁴¹²), rest, diet, salves, and bloodletting were considered the proper treatment for injuries of this important organ. The first case of extirpation of the spleen reported in America is apparently that of O'Brien,³¹⁹ in 1816, for prolapse following a knife-wound. The patient recovered completely in the space of eight weeks.

Another proof that the spleen is not necessary for life is found in occasional reports of congenital absence of

the spleen. In fact, these reports indicate that an individual may live to an advanced age and exhibit no abnormality traceable to the absence of the spleen. The case reported by Hodenpyl¹⁸¹ exhibited a general lymphoid hyperplasia, and it is probable that, as after splenectomy there is a gradual adaptation of the blood-regulating organs, so also, in the congenital absence of the spleen, other organs may take on the function of the missing organ. Since the ten cases collected by Hodenpyl, other authentic cases have been reported by Kohlhass,²²¹ Sternberg,⁴¹⁶ and Riches,³⁷³ making a total of 13. Unfortunately, modern methods of blood examination were not included in the study of any of these cases.

Karl Quittenbaum,³⁶⁸ who introduced the practice of ovariotomy into Germany, was probably the first to plan deliberate splenectomy for disease of the spleen. Numerous successful splenectomies on dogs and cats led him to believe that the omentum, whose vessels were always enlarged, took over the function of the spleen. In 1826 he had occasion to practise the operation on a young woman in an advanced stage of hepatic cirrhosis. Though she suffered from extreme ascites and weakness, he yielded against his judgment to the patient's entreaties, and removed the spleen. She died six hours later from shock.

More important in bringing the operation to the attention of the medical world was another unsuccessful operation, by Küchler²³¹ in 1855. Death occurred a few hours after operation, due to hemorrhage from a branch of the splenic artery that had not been ligated. This gave rise to a lengthy controversy between Küchler and the Verein Hessischer Aerzte, represented by the surgeon, G. Simon. Efforts towards reconciliation by Adelmann, of the Uni-

versity of Dorpat, to whom the matter had been referred, were unsuccessful. On account of Simon's greater reputation, his opinion prevailed that the operation was justifiable only when necessitated by an otherwise fatal wound. Ten years elapsed before splenectomy was again reported, this time in England by Spencer Wells,⁴⁶⁵ whose patient died one week after operation, probably from septicæmia. The nature of the enlargement of the spleen is not stated. In spite of the unsuccessful outcome of this case, attempts at splenectomy quickly became more numerous. Thus Schumann,⁴⁰⁰ collected sixteen cases in 1868; Collier,⁸² twenty-nine in 1882, and Adelmann,⁴ fifty-three in 1887. Since that date the literature on splenectomy has been very thoroughly covered by three authors, Vulpius⁴⁶⁰ (to 1894), Laspeyres²³⁸ (1894 to 1903), and Michelsson²⁹⁰ (1903 to 1913). These reports show that in later years the mortality after splenectomy has been materially reduced. This is due in part to improvement in technic and in part to the general abandonment of the operation in cases of leukæmia, in which condition surgical interference is nearly always disastrous. For the simpler conditions, such as cyst, torsion, or wounds uncomplicated by copious hemorrhage, the mortality is almost *nil*. The recent applications of splenectomy to other conditions, as the anæmias, will be discussed in later chapters.

CHAPTER II

EXPERIMENTAL STUDIES

THE EFFECTS OF SPLENECTOMY IN THE DOG

- (1) THE ANÆMIA, (2) THE INCREASED RESISTANCE OF THE RED BLOOD-CELLS, (3) THE DECREASED TENDENCY OF HÆMOLYTIC AGENTS TO CAUSE HÆMOGLOBINURIA AND JAUNDICE.

The experimental studies here presented had for their object the attainment of new knowledge, and the confirmation or otherwise of older views, concerning the effect produced on the blood by the absence of the spleen. All our experimental observations have been made upon the dog.

The most important changes after splenectomy we have found to be (1) a varying degree of anæmia, (2) increased resistance of the erythrocytes, and (3) lessened tendency to jaundice when hæmolytic agents are administered. Less frequent results which follow the procedure are: (4) destruction of erythrocytes by the endothelial cells of the lymph-nodes and the liver, and (5) transformation of the marrow of the long bones from a yellow to a red marrow. These several changes will be presented, so far as possible, in the order named, and in connection with them will be offered evidence concerning the relation of the spleen to protein, fat, and iron metabolism, and data on other minor phases of the general problem of the relation of the spleen to blood destruction and regeneration. As control studies, a series of observations are offered on the results of diverting the splenic blood from the liver without removal of the spleen.

THE SPLEEN AND ANÆMIA

I. THE ANÆMIA FOLLOWING SPLENECTOMY

This is of the type of the so-called secondary anaemia, characterized by a decrease in the number of red cells and the haemoglobin content, with little evidence at first of regenerative changes. In many instances the anaemia ³⁰⁹ develops almost immediately and progresses gradually until about the end of the first month, when it reaches its point of greatest severity; the return to normal then begins, and a blood condition similar to that before splenectomy

TABLE I * †
RED CELL COUNTS AFTER SPLENECTOMY

Prelimi- nary count	Dog 32	Dog 33	Dog 41	Dog 44	Dog 46	Dog 57	Dog 59
	5,340,000	4,930,000	5,350,000	5,150,000	5,520,000	5,520,000	4,610,000
1*	4,500,000		4,540,000	4,450,000		5,100,000	4,140,000
3	4,700,000		4,710,000	5,090,000		5,120,000	3,690,000
5	4,470,000		4,700,000	4,860,000		5,125,000	2,390,000
7	4,620,000		4,070,000	5,120,000			3,120,000
9	4,800,000		3,920,000	5,060,000		3,940,000	3,960,000
12	4,610,000		3,840,000	4,310,000	5,820,000	3,810,000	4,210,000
15	4,600,000	5,980,000	3,500,000	3,720,000	5,590,000	4,280,000	
18	3,900,000		3,200,000	3,500,000	6,310,000	4,440,000	4,600,000
21				3,390,000		4,390,000	
24	3,550,000		2,890,000		5,760,000	3,150,000	4,460,000
27				3,220,000	5,380,000		3,560,000
30		4,790,000	3,050,000		5,280,000	3,560,000	4,120,000
36	3,720,000			3,100,000	4,960,000	2,970,000	4,510,000
42		3,550,000	2,880,000		4,580,000	3,200,000	4,010,000
48	3,970,000	4,600,000	3,010,000	3,680,000		4,100,000	4,490,000
54	4,240,000	5,020,000	3,260,000			4,210,000	4,570,000
60		4,000,000				4,740,000	
66	4,890,000	4,190,000	3,980,000	4,740,000		5,010,000	4,980,000
72		4,300,000				4,680,000	4,700,000
80	5,350,000	3,680,000	4,950,000	4,740,000		5,980,000	
88		3,860,000				5,320,000	4,340,000
96		4,000,000				5,480,000	5,100,000
104	5,100,000	4,200,000	5,240,000	4,500,000			5,400,000
124	5,230,000	4,340,000	4,610,000	4,150,000		5,240,000	
150		4,800,000				5,216,000	6,250,000
240	6,120,000	5,184,000	5,230,000	4,460,000		5,100,000	5,550,000
300		5,350,000	5,490,000	4,970,000		6,050,000	

* The figures in the first column refer to the number of days after splenectomy.

† In this, as in subsequent tables of blood-cell counts, intervening counts that fail to add to the picture of the blood changes have been omitted.

is reached after two and a half to three months. Rarely the onset³¹¹ of the anaemia may be delayed, and not infrequently the lowest point is not reached for six weeks, and the return to normal delayed until four, five, or six months, or occasionally until even longer periods. In some instances there is an actual rise of haemoglobin and red blood-cell count for several days after operation. This, however, is found after other operations of similar intensity and probably has nothing to do with the removal of the spleen.

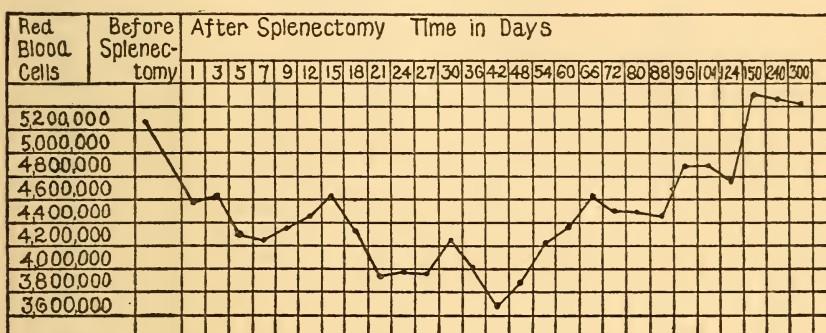


FIG. 1.—Composite curve of the red blood-cell count of seven dogs after splenectomy.

The figures of Table I show in general a prompt onset, a gradually increasing anaemia, and a slow return to normal. An irregularity is seen in Dog 46, which failed to show a fall in the red cell count until one month had elapsed, and also in Dog 44, in which the normal level had not been reached after ten months. Certain minor fluctuations are evident here and there, but in general the anaemia takes a definite course.

As a rule, the decrease in haemoglobin sets in a little later, but is eventually more marked than the fall in the red cell count; also, as improvement begins, the erythrocytes increase more rapidly than does the haemoglobin.

The red cells seldom drop below 3,000,000 or the haemoglobin below 55 per cent. Not infrequently, when the blood picture has returned to normal, the figures are higher

TABLE II
HÆMOGLOBIN ESTIMATIONS AFTER SPLENECTOMY

Prelimi- nary esti- mation	Dog 32	Dog 33	Dog 41	Dog 44	Dog 46	Dog 57	Dog 59
	100 percent.	98 per cent.	90 per cent.	105 percent.	88 per cent.	94 per cent.	80 per cent.
1*	90		70	92		105	70
3	85		70	92		95	64
5	80		70	82		83	48
7	80		70	82			55
9	80		70	72		77	65
12	75		70	72	85	62	65
15	70	88	70	62	98	70	
18	65		70	60	104	77	70
21	65			58		70	
24	65		60		98	71	66
27			50	55	96		63
30		80	55		95	55	74
36	70			57	68	50	70
42		52	55		75	61	80
48	70	58	55	70		76	76
54	80	78	65	72		84	74
60		82				89	
66	90	78	70	75		90	78
72		70				84	75
80	90	79	90	80		82	
88		78				94	72
96		66		90		97	88
104	90	62	85				94
124	95	75	80	95		90	
150		94	85			110	105
240	97	100	75	68		104	105
300		102	92	81		108	

* The figures in the first column refer to the number of days after splenectomy

than before splenectomy. This is true of both the red cells and the haemoglobin. Lamson²²⁵ has recently shown that strong emotions, such as rage or fear, by stimulating adrenalin secretion and thus changing the concentration of the blood constituents, can produce considerable changes in the red blood-cell count per cubic millimetre. In the

animals whose blood counts are here reported, however, various observations indicate that such disturbing factors need not be considered. In the first place, the amount of emotion produced in the process of securing blood counts is slight, temporary, and often almost entirely absent. Furthermore, normal animals have been followed over considerable periods of time without appreciable change in their blood counts.

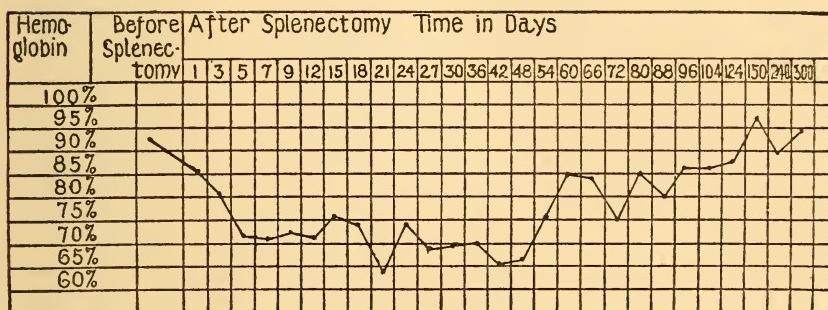


FIG. 2.—Composite curve of the haemoglobin estimation of seven dogs after splenectomy.

The nature of the anaemia that follows splenectomy will be discussed later (see page 87), after some other factors which are dependent upon the removal of the spleen and have a possible relation to the anaemia have been presented. Here it may be stated, however, that the stage of repair is not characterized by the constant appearance in the peripheral blood of nucleated or other abnormal types of red cells. Careful differential counts of the blood of three dogs at regular intervals for 138 days failed to reveal in two the presence of nucleated red cells, and in the other they were demonstrable only five times, the largest number seen in one count being three. In none of

these animals were other changes evident in the red cells, except the pale staining corresponding to a low haemoglobin content. In a large number of other animals in which differential counts were made at irregular intervals, changes in the red cells have been found very rarely. Thus in one dog, five weeks after splenectomy, five normoblasts and one megaloblast were found (in counting 100 leucocytes), as was also evidence of poikilocytosis and polychromatophilia, and, a week later, one normoblast and one megaloblast. These findings were in the first days of beginning repair, the red cells and haemoglobin having a few days before reached the lowest level observed during the experiment; haemoglobin, 50 per cent.; red cells, 2,970,000. In another dog, two months after splenectomy, again at the stage of beginning repair (haemoglobin, 62; red cells, 3,650,000), five nucleated red cells were found, and polychromatophilia was evident. In no instance did these findings persist for any length of time. They are of significance only in that they probably indicate the period of beginning repair.

The very definite nuclear particles found by Morris,³⁰⁰ Roth,³⁸⁵ and Gilbert¹⁴⁵ in the red cells of man after splenectomy we have not observed in the dog, despite repeated examinations made since Morris's second publication. This question of repair of the blood will be discussed again (see page 140) in connection with the study of changes in the bone-marrow.

The number of skeined or reticulated erythrocytes is very slightly, if at all, increased during the early stages following removal of the normal dog's spleen. Gates,¹³⁷ on the other hand, has shown that there is an increase in the number of reticulated cells when the anaemia is greatest;

that is, just before the period of beginning repair. The blood-platelets also are only slightly increased after removal of the normal spleen.

WHITE CELLS.—As our problem was one concerning the red rather than white cells, the latter have not been studied as thoroughly as have the red cells. We have, however, total leucocyte counts of five dogs and differential counts of three dogs, in each instance covering long periods of time,³¹¹ and on many others for shorter periods. The results of the total leucocyte counts are presented in Table III and Fig. 3, and of the differential counts in Table IV.

TABLE III
LEUCOCYTE COUNT AFTER SPLENECTOMY

Preliminary count	Dog 32	D g t 1	Dog 44	Dog 57	Dog 59
	9,000	12,000	13,000	14,400	14,200
1*	26,000	33,000	36,000	38,100	28,700
3	26,000	22,500	21,500	21,100	13,800
5	28,000	21,000	13,000	17,000	11,400
7	25,500	23,000	13,000		17,900
9	28,000	19,000	14,000	22,000	14,400
12	22,000	21,000	13,000	19,200	17,800
15	25,000	18,000	16,000	19,600	
18	19,000	19,000	18,100	18,700	16,800
21	18,000	14,000	16,400	18,400	
24	15,000			18,600	12,100
27		15,000	12,000		11,800
30	33,000	15,000	14,200	16,000	13,400
36				16,900	15,300
42	16,000	11,000	11,200	19,000	12,100
48		12,000		19,200	17,400
54	11,000	11,000	10,000	15,000	13,100
60				16,100	
66	13,000			17,600	11,600
72		12,000	11,200	16,400	12,200
80	14,000	12,000		16,000	
88			9,900	18,100	13,600
96	13,000	13,000	11,000	20,100	9,000
104					
124	15,000	13,000	12,400	16,000	
240				16,400	15,000

* The figures in this column refer to the number of days after splenectomy.

The leucocyte picture was quite constant. On the day after splenectomy the white cells rose from a normal level of 9000–14,000 to 38,000 per cubic millimetre, or even higher, and in a few days fell rapidly to about 20,000, after which there was a more gradual decrease with return to approximately the normal level after a period ranging from one to four months. The initial leucocytosis was due mainly to an increase in the polymorphonuclear neutrophilic leucocytes, and was probably a post-operative effect

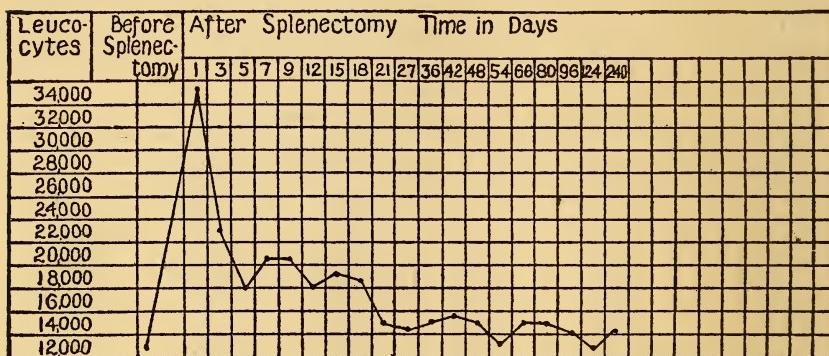


FIG. 3.—Composite curve of the leucocyte counts of five dogs after splenectomy.

and not due to the specific absence of the spleen. Lymphocytosis, although usually described, has been found only to a slight degree in this series. At no time were the lymphocytes recorded as higher than 34 per cent., while the average count in all dogs was about 18 to 26 per cent. In the parallel observations on the results of diverting the splenic blood from the liver without removal of the spleen, the early polymorphonuclear leucocytosis and later lymphocytosis were also noted. The behavior of the eosinophiles has varied; in two dogs (not presented in Table IV) an eosinophilia of 10 to 32 per cent. persisted for 113 days in one, and in the other an eosinophilia of 6 to 11

EXPERIMENTAL STUDIES

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TABLE IV
DIFFERENTIAL COUNTS OF WHITE CELLS AFTER SPLENECTOMY
Dog 1

Time	Leucocytes	Polynuclears	† Lymphocytes	† Eosinophiles	† Other forms
Before operation	12,500	8,625	3,000	500	375
*1	45,200	37,100	5,880	450	1,770
3	22,400	17,250	3,360	560	1,230
7	25,600	18,700	4,340	1,280	1,280
13	25,100	19,500	4,100	1,000	500
21	18,600	13,580	4,460	370	190
33	17,100	11,950	4,360	0	790
47	14,500	10,140	4,000	0	360
68	13,600	9,590	3,460	0	550
82	14,700	11,250	2,860	150	440
96	13,100	9,560	2,360	785	395
111	12,700	8,000	3,230	700	770
138	15,900	9,700	2,380	2,070	1,750

Dog 2

Time	Leucocytes	Polynuclears	† Lymphocytes	† Eosinophiles	† Other forms
Before operation	10,100	7,770	1,615	200	515
*1	48,400	43,570	2,905	240	1,685
3	25,100	19,300	3,890	1,510	400
7	23,100	17,750	4,430	230	690
13	18,200	13,650	3,640	90	820
21	16,800	12,600	3,700	0	500
33	15,100	11,180	3,470	0	450
47	12,600	9,350	2,750	0	500
68	11,400	8,780	2,280	0	340
82	12,600	9,340	2,700	0	560
96	11,900	8,360	2,610	360	570
111	11,800	8,550	1,770	760	720
138	16,100	11,110	1,770	1,610	1,610

Dog 3

Time	Leucocytes	Polynuclears	† Lymphocytes	† Eosinophiles	† Other forms
Before operation	12,500	8,650	3,380	190	280
*1	26,900	21,480	3,500	1,070	850
3	21,100	15,750	4,720	210	420
7	12,200	8,800	2,650	250	500
13	16,100	11,350	4,520	0	230
21	14,100	9,850	3,590	0	660
33	14,100	10,860	2,820	0	420
47	12,100	8,660	3,200	0	240
68	12,000	8,400	3,360	0	240
82	10,300	7,420	2,680	0	200
96	13,100	8,540	3,530	450	580
111	13,700	9,040	3,560	410	690
138	13,800	9,660	1,930	410	1,800

* Numbers in the first column indicate the number of days after operation; numbers in the other columns indicate the number of cells per cubic millimeter.

† Calculated on basis of 200 cells counted.

per cent. for 107 days. On the other hand, in three dogs the eosinophiles disappeared entirely for long periods of time, corresponding roughly to the third to eleventh week,* but this disappearance was followed later by an increase varying from 6 to 20 per cent. of the total white cell count. This disappearance, as may be seen in Table IV, corresponded to the point of severest anæmia (thirty-third, twenty-first, and thirteenth days), and the reappearance to the period when the blood has returned, or nearly so, to its normal level (eighty-second, ninety-sixth, ninety-sixth day). As to its significance we have no opinion.

In all differential counts especial search has been made for unusual cells, as myelocytes. These have seldom been found. In the counts shown in Table IV, representing three different dogs, basophilic myelocytes were found only four times, and no other atypical leucocytes were seen. In a fourth animal, two months after splenectomy, basophilic myelocytes (5 to 100 cells) were found once at the period of beginning repair.

The literature of experimental splenectomy, while it presents rather widely varying results, is, on the whole, in accord with our experience. Dissimilar results are reported by Paton, Gulland, and Fowler,²³⁰ who state that in the dog, cat, and rabbit the removal of the spleen has no influence on the red corpuscles. An examination of their tables shows, however, that in the dog they did obtain a slight anæmia, a decrease of 600,000 to 800,000 red cells, which, however, occurred also in one instance in a normal dog. Moreover, they used puppies about two and a half

* In one of our papers²¹¹ in which we refer to this phenomenon, the period is given as "the third to the eleventh day." This is incorrect and should read "week" instead of "day."

months old, and it is possible that in such young animals the mechanism of blood destruction and regeneration may not be the same as in older dogs. Our observations were mostly upon full-grown dogs, but in the few puppies studied the results were substantially the same. Other results not in accord with ours are those of Azzurrini and Massart,²⁴ who made frequent counts on four dogs over periods of 15 to 18 months after splenectomy. In none was a drop of more than 5 per cent. haemoglobin or 100,000 red blood corpuscles noted. In this connection we may point out that where anaemia fails to develop, the possibility of accessory spleens which are sometimes found in the dog must be considered. Zanda,⁴⁸⁴ for instance, failed to find the usual changes after splenectomy, and states that this was due to the presence of accessory spleens. Diet, as we will show later, also has an important influence on the degree of anaemia. Wolferth⁴⁷⁷ has recently shown that the same changes occur in the blood of Albino rats after splenectomy that we have found in dogs. In eight rats which had abnormally large spleens, splenectomy was followed by rapid severe anaemia, hyperleukocytosis, marked increase in the number of nucleated and reticulated cells, and proved fatal in seven of the eight.

Picard and Malassez,³⁵² by the crude methods of early blood examination, found a diminution in both red cells and haemoglobin, but considered the latter to be more definite. Vulpius,⁴⁶⁰ Gibson,¹⁴⁰ Laudenbach,²⁴⁰ Grigorescu,¹⁵⁵ Winogradow,⁴⁷⁵ and Tauber⁴²⁸ record a decrease in red cells after splenectomy in the dog, and Vulpius describes a leucocytosis, as does also Gibson. The degree of anaemia, as well as its severity and the time of repair as observed by these different investigators, varies widely,

but they are in general accord as to both anæmia and leucocytosis. That anæmia occurs in other animals than the dog is shown by Warthin's⁴⁶¹ observations on the sheep and goat. On the other hand, the changes in the rabbit and guinea-pig are not so uniform. Gabbi¹³⁵ found in guinea-pigs an increase in red cells and haemoglobin, and in the rabbit no change or a slight decrease, as did also Zezas.⁴⁸⁵ In the rabbit and cat, Paton, Gulland, and Fowler³³⁰ found no changes. Asher and Sollberger¹⁷ have recently stated that in the rabbit removal of the spleen causes an increase of both red cells and haemoglobin, and that this increase is due to the removal of the normal haemolytic activity of the spleen and to stimulation of the bone-marrow. Their observations, however, were made only a short period after splenectomy, so that the rise noted may be similar to that occasionally found by us in dogs immediately after splenectomy. Most of the observations concerning the anæmia following splenectomy are, in fact, incidental to other problems or are based on occasional examinations, which perhaps accounts for some of the discrepancies. We feel, however, that in the large number of animals which we have studied it is conclusively shown that a secondary anæmia of some degree is, in the dog at least, a characteristic result of splenectomy. The variations in the degree of anæmia are, however, so marked that we have made a prolonged study of the influence of diet³³⁷ in the hope of explaining these variations.

Influence of Diet on the Anæmia.—In these studies we had in mind: (1) the observations of Asher and Vogel,¹⁸ that while an iron-poor diet (sugar, starch and lard) has no effect upon the blood picture in a normal dog, in the splenectomized dog on the same diet, a great decrease

in number of red cells and amount of haemoglobin occurs; and further, if under the latter circumstances an iron-rich (flesh) diet is given, the blood picture quickly returns to normal; (2) Richet's³⁷⁴ observation that in order to maintain splenectomized dogs at the same weight as normal dogs, a much larger quantity of food is necessary, and (3) Paton's³²⁹ conclusion opposed to that of Richet, that splenectomy in the dog has no influence upon general metabolism.

In regard to Asher and Vogel's contention, we have not found by a direct quantitative study (see page 112) of the elimination of iron that splenectomy seriously influences iron metabolism.²² Moreover, in our opinion, the time of improvement in the anaemia which these investigators describe as the result of feeding iron-rich food corresponds to the spontaneous repair of the anaemia which usually begins about the end of the fourth week. In other words, the improvement was, in our opinion, due in part at least to the normal repair and not to the effect of the iron-rich food. Their conclusions would be more convincing if they had prevented entirely, or lessened, the severity of the anaemia by beginning the feeding immediately after splenectomy instead of waiting nearly three weeks. As to Richet's point, it may be noted that we have not seen noteworthy changes in the weight of our splenectomized animals. For a few days after splenectomy, a slight loss may occur, but in all long time experiments an increase beyond the original weight has been observed.

The studies of Paton and his associates as to the changes in the blood after splenectomy are the most carefully conducted of any in the literature and for this reason we have been greatly disturbed that our results were so differ-

ent. Their studies, however, were limited to two splenectomized animals and two controls and it may be that by chance the former correspond to the milder anæmias which we observed. In their studies of the blood³³⁰ diet is not mentioned, but in the metabolism³²⁹ work the dogs were for part of the time at least on a meat (high iron) diet, which, if used in the blood work also, might have been, if Asher and Vogel are correct, a factor in decreasing the anæmia. It is evident from this brief review that diet may be an important factor in determining the degree of anæmia following splenectomy.

Until our special investigations of the influence of diet were undertaken, all animals, except those used in the study of iron metabolism,²² had been kept upon the same general diet—a mixture of meat, bread, cereals and vegetables—in all essentials, the “table scraps” upon which dogs are usually fed. This was always supplied in abundance and each dog received all he would eat, and as our records show that the splenectomized dogs during periods of several months gained in weight on this diet, we considered it highly satisfactory. However, we did not know the exact caloric value of this mixed diet and, moreover, as it was essentially a boiled diet, it might possibly be deficient in some substance essential to the proper function of the hæmopoietic system. Therefore, in our first group of dietary experiments animals were placed on calorically sufficient diets, the protein being furnished in the form of beef heart, beef spleen, or commercial casein, and the fat and carbohydrate in the form of lard and bread crumbs. Beef spleen was introduced on account of its large iron content in contrast with that of the beef heart and the casein. Several blood examinations were made during a

period of ten days to two weeks before splenectomy and at intervals, never exceeding a week, after operation. In Tables V, VI, and VII, which show the results of these studies, only the last two blood counts of the preliminary periods are given. These represent, usually, counts made respectively 1 to 2 and 5 to 7 days before splenectomy. As the blood of the several dogs was not always examined at exactly the same intervals after splenectomy, in

TABLE V
INFLUENCE OF DIET

Days	Raw beef heart, lard, and bread						Days	Raw beef heart, lard, and bread			
	Dog 79 (splenectomized)			Dog 83 (splenectomized)				Dog 81 (control)			
	Weight	Red cell count	Hæmoglobin	Weight	Red cell count	Hæmoglobin		Weight	Red cell count	Hæmoglobin	
Before splenectomy											
	kilos.	7,930,000	percent.	kilos.	7,000,000	percent.	1	9.1	6,320,000	percent.	
	9.3	7,560,000	110	8.7	7,770,000	107	4-7		6,650,000	102	
			114			105	12-18		6,880,000	96	
							26-40	10.0	6,800,000	90	
							40-60	10.1	6,000,000	96	
After splenectomy											
5-7	8.8	7,420,000	97	8.6	7,910,000	105					
10-14		7,230,000	98		7,720,000	103					
18-23		6,250,000	98	8.6	6,500,000	96					
26-33		6,810,000	96	8.7	7,270,000	101					
38-40	9.8	7,360,000	104	8.3	6,880,000	97					
45-48	10.5	6,690,000	100	8.5	6,260,000	96					
52-61	10.8	6,640,000	95	8.6	6,240,000	93					

order to shorten the table, only enough blood counts are given to show the general trend of the blood picture. The figures for iron and of nitrogen in the diet are based on the average of several estimations of the food materials used. These figures with a calculation of the caloric value of the food are given in Table VIII.

By comparing Tables V, VI and VII it is at once evident that in no instance did the general nutrition of the animals suffer. A slight loss of weight occurred after operation, but this was soon regained. Also it is seen that

in no instance does a splenectomized dog maintain the same constant level of red cell and haemoglobin content as do the non-splenectomized animals. The change, however, in Dogs 79, 83, 84 and 87 is so slight as to be within the limit of error of the methods of blood examination; in 82, 85 and 86 the change is more marked, but even here one can hardly refer to the condition present as a frank anaemia. It is, however, of significance that in all instances the varia-

TABLE VI
INFLUENCE OF DIET

Days	Casein, lard, and bread						Days	Casein, lard, and bread			
	Dog 82 (splenectomized)			Dog 84 (splenectomized)				Dog 80 (control)			
	Weight	Red cell count	Hæmoglobin	Weight	Red cell count	Hæmoglobin		Weight	Red cell count	Hæmoglobin	
Before splenectomy											
	kilos.	5,500,000	percent.	kilos.	7,780,000	percent.	1	kilos.	7,860,000	percent.	
	16.7	5,551,000	98	8.7	7,710,000	98	4-7	9.1	7,420,000	90	
			85			97	12-18		7,020,000	92	
							26-40	8.3	7,370,000	96	
							40-60	9.2	7,180,000	96	
After splenectomy											
5-7	16.5	4,600,000	75	8.6	7,090,000	89					
10-14	17.2	4,688,000	65	8.8	7,140,000	92					
18-23		4,210,000	76	8.9	6,600,000	91					
26-33		5,160,000	82	9.1	7,010,000	92					
38-40		6,040,000	88	9.2	6,940,000	87					
45-48					6,630,000	91					
52-61	16.9	6,060,000	90	9.1							

tions are more marked than in the controls and also that they usually occur after about four weeks, the period, in post-splenectomy anaemia, usually marked by the lowest counts. On the other hand, the question arises, are these results in some way due to the diet, that is, to the general character of the diet, or to the presence of large amounts of iron? That iron in the diet is a factor seems doubtful in view of the fact that two of the three animals (85 and 86), fed with beef spleen, showed the most marked changes of any in the group. Beef spleen was selected because it

TABLE VII
INFLUENCE OF DIET

contains a large amount of iron, according to our analyses 235 mg. per 100 grams, presumably in large part in organic combination and therefore readily utilizable. Fresh beef heart and casein, on the other hand, contain only 4.6 mg. and 7.2 mg. per 100 grams respectively, and if iron is an important factor in preventing anæmia after splenectomy,

TABLE VIII
NITROGEN AND IRON CONTENT AND CALORIC VALUE OF DIETS OF TABLES
V, VI, VII

Dog No	Actual total per day			Per kilo of body weight per day		
	Nitrogen <i>gm.</i>	Iron <i>mg.</i>	Calories	Nitrogen <i>gm.</i>	Iron <i>mg.</i>	Calories
79	6.5	11.8	709	0.72	1.31	79
83	9.8	18.0	1,043	1.17	2.15	124
81	6.5	11.8	665	0.68	1.23	69
82	13.6	11.3	1,194	0.81	0.68	71
84	10.6	9.1	956	1.18	1.01	106
80	7.4	6.2	664	0.82	0.69	74
85	4.6	352.0	517	0.69	53.00	77
86	8.3	653.4	893	0.69	54.00	74
87	6.8	543.0	752	0.68	54.00	75
90	4.6	352.0	517	0.64	49.00	72

one would not expect animals fed with spleen to show the changes evident in the figures given for Dogs 85 and 86; rather, one would expect figures as in experiment 87. The changes in these three animals, in all probability, represent the variation to be expected in any group of animals. That the administration of abundant organic iron in the form of beef spleen, did not prevent the anæmia, is in accord with our studies²² of iron metabolism* in the absence of the spleen and opposed to the conclusion of Asher and Vogel.¹⁸

On the other hand, in view of the slight changes which

* See page 112.

occurred in some of the animals, it is impossible to avoid the question as to whether a diet adequate for the normal dog is in some way inadequate for the splenectomized dog. If the latter could be demonstrated the value of our views concerning the severer types of anaemia following splenectomy, based on our earlier experiments upon dogs fed on a general mixed diet, would depend upon whether or not the inadequacy of diet held for all animals operated upon, or only for animals without a spleen. If anaemia occurred in dogs fed on the mixed diet after other operations than splenectomy, it would be at once evident that the food, while sufficient for a normal dog, was not sufficient for a convalescent dog. On the other hand, if the anaemia could be demonstrated only after splenectomy, there would be established a point of importance in regard to the spleen in its relation to metabolism, and our observations on the anaemia after splenectomy would not only be substantiated, but would gain an added importance. To settle this point, it was essential, therefore, to study in animals on our routine mixed diet the effect of splenectomy, and as a control some other simple operation involving the removal of an organ. Nephrectomy was selected as an operation quite analogous, from the technical point of view, to splenectomy, and accordingly two healthy dogs were placed upon ordinary kennel diet for seventeen days; upon each dog a nephrectomy was then performed and the animals kept on the same diet for twenty-three days longer; splenectomy was then performed upon each dog and the animal kept on the same diet for thirty-eight days more. Blood counts were made at frequent intervals throughout the experiment. Whereas during the seventeen days on the diet before operation and during the twenty-three days follow-

ing nephrectomy no significant change (see Table IX) in the haemoglobin or red blood-cells was observed in either animal, after the splenectomy both showed a well-marked fall in haemoglobin and red blood-cells count. It is noteworthy, also, that relatively slight change in weight occurred.

TABLE IX
EFFECT OF SPLENECTOMY CONTROLLED BY PREVIOUS NEPHRECTOMY. MIXED DIET

Date	Dog 23			Dog 42		
	Weight <i>kilos.</i>	Hæmo- globin <i>per cent.</i>	Red blood count	Weight <i>kilos.</i>	Hæmo- globin <i>per cent.</i>	Red blood count
May 18....	10.9	100	6,840,000	14.1	102	7,810,000
May 25....	10.9	98	7,240,000	14.0	102	7,850,000
June 1....	11.0	92	6,760,000	14.9	101	8,330,000
June 4....	Nephrectomy			Nephrectomy		
June 8....	11.1	93	6,280,000	14.0	98	7,210,000
June 15....	11.3	90	6,020,000	14.6	95	7,120,000
June 25....	11.4	97	6,690,000	14.8	97	7,010,000
June 27....	Splenectomy			Splenectomy		
July 3....	10.9	90	6,600,000	15.1	90	6,840,000
July 10....	10.8	83	5,890,000	14.9	92	6,830,000
July 18....	10.8	77	5,740,000	14.9	88	6,480,000
July 27....	10.4	66	5,080,000	14.3	75	6,060,000
Aug. 4....	10.4	68	4,540,000	14.2	76	6,350,000

From these observations four conclusions may be drawn: (1) that, inasmuch as the animals maintained their average weight, the routine "table scrap" diet is a satisfactory food for animals after surgical operations; (2) that on this diet, operation involving the removal of an organ other than the spleen does not cause anaemia; (3) that the anaemia following splenectomy is not to be explained, in view of the fact that the splenectomized animals maintained their average weight, by insufficient nutrition;

and (4) if the anaemia is in any way related to the diet, it is either (*a*) because some toxic substance, which operates in the absence of the spleen, is present in this particular food, or (*b*) because some substance present in the diet and normally utilizable cannot be utilized in the absence of the spleen. In connection with this last conclusion, it occurred to us that, as the routine kennel diet is essentially a cooked diet, it was possible that in the cooking there occurred the destruction by heat of some vitamin-like substance normally utilized by the spleen. To control this

TABLE X

THE INFLUENCE UPON THE ANAEMIA FOLLOWING SPLENECTOMY OF A RAW AND A COOKED DIET

Dog No.	Diet	Before splenectomy				After splenectomy				Food values per kilo. of body weight	
		Period	Weight before operation	Hæmoglobin	Red cell count	Period	Final weight	Hæmoglobin	Red cell count	Nitrogen gm.	Calories
48	Raw	days 28	kilos. 13.4	per cent. 99	5,450,000	weeks 6th- 9th	kilos. 14.0	per cent. 96	5,590,000	0.41	69
57	"	25	10.9	100	6,220,000	6th-12th	12.4	83	4,499,000	0.46	75
53	"	24	8.0	99	6,140,000	8th-12th	8.9	75	4,920,000	0.74	69
52	"	41	8.5	104	6,910,000	10th-13th	8.6	83	5,551,000	0.15	72
52	Cooked	48	10.8	105	6,760,000	7th- 9th	11.4	77	5,130,000	0.40	69
56*	"	121	8.4	88	6,250,000	6th-10th	7.8	61	4,880,000	0.40	73

Controls

9	Raw	108	11.4	102	6,620,000 (initial period)						
				12.9	95	6,590,000 (final period)					
56*	Cooked	121	8.2	100	6,460,000 (initial period)						
				8.4	88	6,250,000 (final period)					

* This animal was used first as a control for the cooked diet and later was splenectomized.

point (see Table X) a new series of observations were undertaken. Six animals were placed upon a calorically sufficient diet, accurately determined, the only difference being that four received raw and two cooked meat. Examinations of the blood were made at intervals of not longer than seven days. At the same time metabolism studies, the results of which are described elsewhere,* were made on some (No. 48, 57, 52, and 56) of the animals. The diet in each of these experiments consisted of beef-heart, lard, and sugar, a small amount of sodium chloride, and sufficient bone-ash to ensure firm faeces. Details of nitrogen content and caloric value of the foods are given in Table X. In connection with this table, it should be explained that, in order to place the figures covering all animals in one graphic table, the counts given represent averages of several examinations. The figures before splenectomy represent the averages of the last three counts before operation; the figures after splenectomy, the average of the three lowest consecutive counts. The figures for the two control animals represent the average of the first three and last three counts respectively.

It is evident, from a study of this table, that there is a greater tendency for animals on the cooked diet to develop anaemia than is the case with those receiving raw meat. Thus in the latter group no change in the blood picture was evident in one animal, while in the other three with moderate anaemia the haemoglobin did not fall below 75 or the red cells much below 5,000,000. On the other hand, both animals receiving cooked meat showed a marked change in the blood picture, and in one a haemoglobin content as low as 61. The haemoglobin decrease is relatively

* See page 181.

greater, as in all our previous studies, than is the fall in red cells. That the amount of protein given in the raw food is not an important matter is seen by contrasting Dog 53 on a high nitrogen diet with Dog 50 on a low nitrogen diet. In these two animals the calories of the diet were maintained by varying the amount of fat. The difference in the degree of anaemia is negligible. In connection with the problem of the influence of cooked diet, it is noteworthy that Dog 56, which served as a control to 52 for four months before it was splenectomized, and was living all this time on a cooked diet, showed during this time a falling off in the haemoglobin content of its blood. Moreover, this animal was the only one showing a persistent loss of weight after splenectomy. Definite conclusions cannot be drawn from such a small number of experiments, but the fact that splenectomized animals on cooked beef develop an anaemia of a degree more closely approaching that of animals on the usual kennel diet, essentially a cooked diet, while animals on a raw diet have a less severe anaemia, suggests that heat brings about some change in the diet, which in the absence of the spleen is a factor in causing anaemia. In view, however, of the relatively slight differences which we have found, experiments on a large number of animals on diverse diets must be made before a final decision can be reached on this point. Our views at present may be summarized as follows:

1. The anaemia which develops after splenectomy is most marked in animals on a mixed "table scrap" diet of meat, bread, cereals, and vegetables, which is essentially a cooked diet.
2. Control studies in which a unilateral nephrectomy precedes splenectomy demonstrates that the anaemia is

not due to operation, hemorrhage, or accidents of convalescence, but develops only in the absence of the spleen.

3. The results of studies of the influence of food containing a large amount of iron in presumably easily utilizable form, as in raw beef spleen, does not support the view that the anæmia is due to lack of iron in the food.

4. Observations on the influence of a diet of raw meat as contrasted with cooked meat show a more severe anæmia in animals on the cooked diet, and suggest the possibility that heat alters some substance which the body cannot utilize in the absence of the spleen.

BLOOD CHANGES IN MAN AFTER SPLENECTOMY

Concerning the influence of splenectomy in man, a wide difference of opinion exists. This is reflected in the text-books on haematology; for example, DaCosta recognizes a diminution of red cells and haemoglobin and a leucocytosis, the former continuing one to three months, the latter four to six weeks. Cabot does not mention changes in the red cells or haemoglobin. Grawitz says there is no especial change in the red cells. All these authors, however, recognize a lymphocytosis and an eosinophilia.

It is not difficult to understand this difference of opinion. The spleen is usually removed for some acute traumatic condition, as rupture, or some chronic splenomegaly associated with anæmia, in all of which conditions the blood picture is altered. Frequently, also, no preliminary blood examination is made, and if such examination is made, observations are not continued long after operation. Under these conditions it is not surprising that opinions should vary. Accurate information concerning the influence of the spleen must be based on extirpation, for

some localized lesion which replaces or affects only a small amount of the organ, does not alter the function of the remainder, and has in itself no influence on the blood picture. When the spleen is removed under such conditions, as in a simple cyst, or for misplacement, or for injury without massive hemorrhage, the blood picture being normal before operation, definite conclusions concerning the effect of splenectomy may be reached. A few instances of this kind exist in the literature. For instance, Darling,⁸⁸ before the removal of a simple non-parasitic cyst of the spleen, found the hæmoglobin to be 90 per cent. and the red blood-cell count on two occasions 4,290,000 and 4,750,000, respectively. The day after a relatively simple operation, without hemorrhage, the red blood-cell count had dropped to 3,937,000, and three days after operation to 3,280,000. Three weeks later the hæmoglobin had returned to 100 per cent. and the red blood-cells to 4,700,000. Dr. Darling has been good enough to write us that three years after operation the blood examination was as follows: Hæmoglobin, 100 per cent.; red blood-cells, 5,300,000; and leucocytes, 10,000, of which polymorphonuclears form 75 per cent., small lymphocytes 12 per cent., large mononuclears 5 per cent., and eosinophiles 8 per cent.

Catellani,⁶⁷ in a preliminary count before extirpation of a movable spleen, found hæmoglobin 70 per cent. and red blood-cells, 4,435,500. One month after operation the hæmoglobin had dropped to 60 per cent. and the red blood-cells to 3,500,000. After ten weeks the hæmoglobin had returned to 75 per cent. and the red blood-cells to 6,050,000; white cells, 28,310. Meyers²⁸⁷ reports a child of eleven years splenectomized three days after an injury causing rupture of the spleen. On the evening of operation

the blood picture was: Red cells, 5,250,000; white cells, 28,000. Within twenty-four hours the red cells fell to 3,030,000, and at the end of twelve days to 2,980,000. The haemoglobin figures (Tallqvist) offer little of value: Great variations in the red cell count were observed for three months with gradual improvement, but not complete return to normal in that time.

McCoy²⁶⁰ reports two cases of rupture, one of a normal, the second of a malarial spleen. In the first, on which seven counts were made, the haemoglobin fell from 70 per cent. before operation to 20 per cent. three days after operation and steadily rose to over 100 per cent. sixteen months after operation. The red blood-cells numbered 3,408,000 before operation, fell to 2,300,000, and steadily rose to 5,560,000. The patient with malarial spleen showed haemoglobin 70 per cent., red blood cells 4,000,000 before operation, with a fall to 35 per cent. and 2,496,000 immediately after the operation, and a rise to 90 per cent. and 5,456,000 seven months later.

In the case reported by Matthew and Miles²⁷⁸ a count made three days after splenectomy for rupture showed 3,800,000 red blood-cells and 80 per cent. haemoglobin, and after two years, 4,800,000 red blood-cells and 85 per cent. haemoglobin.

Heaton¹⁷² reports a splenectomy for traumatic rupture in a child nine years of age. The operation was done five and one-half hours after the injury, when serious symptoms of internal hemorrhage had developed. No preliminary or early counts were made—a count seven days after operation showed 4,100,000 red cells and haemoglobin 40 per cent.; after five months of otherwise perfect recovery the blood picture was 4,480,000, haemoglobin 75 per cent. The usual leucocytosis was observed.

Fowler,¹²⁶ a few hours after splenectomy for movable spleen, found the red cells to number 3,900,000 and the leucocytes 34,200. Daily counts thereafter for twenty-seven days showed a gradual decrease in the red cells, with a slight increase at the end of that time to 4,147,000, the white cells at the same time being 12,880. Unfortunately, no blood examination was made before operation.

Musser's³¹⁰ case of cyst of the spleen illustrates well the degree of anaemia which may occur and the long duration

TABLE XI
BLOOD EXAMINATIONS BEFORE AND AFTER REMOVAL OF THE SPLEEN FOR SIMPLE CYST

	Hæmo-globin	Red blood cells	Leuco-cytes	Polymor-phonu-clears	Lym-phocytes	Mono-nuclears and transi-tionals	Eosin-ophiles	Baso-philes
Before operation	70	4,360,000	11,400	7,410	3,310	340	340	0
8 days after	90	4,642,000	11,400	8,050	1,660	1,390	270	30
18 days after	87	3,916,000	13,900	8,340	3,340	2,220	0	0
9 months after..	80	4,400,000	17,000	11,950	4,250	700	100	0
15 months after..	75	4,220,000	14,800
22 months after..	75	2,750,000	12,600
25 months after..	80	3,460,000	13,200
28 months after..	78	3,400,000	14,800
40 months after..	80	3,590,000	17,200	9,370	7,310	520	0	0

of the same. The patient was a young woman, twenty-five years of age, with, for seven years, a swelling in the left side of the abdomen. At operation was found a large benign cyst of the spleen measuring 18 cm. in diameter. The spleen with cyst wall (after evacuation) weighed 400 grammes, so that, exclusive of the cyst, the spleen itself was not greatly enlarged. In the detailed statement of the blood examinations, presented in Table XI, it will be seen that a slight anaemia existed before operation.

Staehelin^{413 (a)} has collected from the literature up to 1903 twenty-one cases of splenectomy following injury

and constituting, therefore, removal of the normal spleen. In very few of these cases was preliminary examination of the blood made, and in only three instances was the blood examined more than three times, and usually at very irregular intervals. In all cases there is evidence of some grade of anæmia after operation, but the counts were not sufficiently numerous to allow of definite opinion as to the general course of the anæmia. The figures as to leucocytosis vary greatly.

These few records presented in detail have been selected from a large number of reports, most of which, because they present no preliminary counts or only occasional counts, are of little value in furnishing reliable evidence of the influence of splenectomy on the blood picture. Such evidence as we have shows very distinctly that in man both red cells and haemoglobin decrease, the latter more than the former, after splenectomy. Some confusion exists because of the improvement in the blood picture that follows operation for removal of the enlarged spleen of the various types of "splenic anæmia." In these cases, however, an abnormal spleen is removed under abnormal conditions of the blood, and not a normal spleen under normal conditions, and, as we will show later, several factors are to be considered in connection with the changes in the blood following removal of the pathological spleen.

II. INCREASED RESISTANCE OF RED BLOOD CELLS

Early in our work with splenectomized animals it was found that they are more resistant to haemolytic poisons than are normal animals. This fact had previously been observed in the dog by Bottazzi,⁵³ Banti,²⁸ Pugliese and Luzzatti,³⁶⁴ and Joannovics,²⁰⁰ while Domenici⁹⁵ found it

true also of the rabbit. Evidence of the increased resistance was based on the fact that to get the full toxic effect of a haemolytic poison it was necessary to give a splenectomized dog doses two or three times greater than were required for the normal dog. As a result of such observations the theory was advanced that the spleen was concerned in some way in influencing the normal destruction of aged and effete erythrocytes, and that, as this influence was lost after splenectomy, haemolytic agents were correspondingly less effective. This was the basis of Bottazzi's theory of the spleen as a haemocatatonistic (*i.e.*, preparing red blood-cells for destruction) organ, which was later adopted by Banti and supported to some extent by Joannovics. Pugliese and Luzzatti,³⁶⁴ on the other hand, denied the influence of the spleen, and pointed to the presence, in experimental anaemia due to pyrodin, of newly-formed immature corpuscles, poor in haemoglobin and frequently nucleated, which they thought might be more resistant to pyrodin than were the normal corpuscles and in this way responsible for the lesser degree of haemolysis.

The first definite demonstration of a change in the red cells was made by Bottazzi, who, experimenting on dogs, found that the red cells after splenectomy showed an increased resistance to haemolysis in hypotonic salt solutions. This increase first appeared a few days after operation, and increased gradually up to a certain point, where it stayed indefinitely. He considered three possibilities: that the increased resistance was due (1) to the anaemia following splenectomy, (2) to more resistant young cells from a rapidly proliferating bone-marrow, (3) that the spleen has a special function to weaken certain red blood-cells. From various controls he deduced that the last

theory was correct, and upon this based his theory of the spleen as a hæmocatatonistic organ.

At about the same time Domenici⁹⁵ showed that under similar circumstances the erythrocytes of the rabbit possessed an increased resistance. It is only within the last five or six years, however, that definite measurements of the resistance of the corpuscles have been made and thereby the degree of increased resistance clearly established.

Brissaud and Bauer,⁵⁰ working with two rabbits and using hypotonic solutions of varying strengths, found a decreased resistance of the red cells during a period of eight to ten days after splenectomy, with, after this lapse of time, a return to normal but no increase in resistance; they did not, however, continue their tests for periods of more than ten days. Similar negative results were obtained by Biagi⁴⁵ in dogs. Chalier and Charlet,⁷¹ testing both the rabbit and the dog by the salt solution method, concluded that splenectomy is followed by a slight increase in the resistance of the red cells.

Pel,^{340a} in a very extensive study, found as an average of fifty-eight determinations that in normal dogs the first trace of hæmolysis occurred in 0.42 per cent. salt solution, as compared with 0.35 as the average for thirty observations on splenectomized dogs; the average concentration at which hæmolysis was complete was, for normal dogs, 0.30 per cent. salt solution, and for splenectomized dogs 0.23 per cent. Thus in both series of observations the increased resistance of the splenectomized animals was the equivalent of 0.07 per cent. salt solution. The difference may be expressed in another way: in the forty-eight observations on normal animals hæmolysis began in all except one test in solutions of 0.48 to 0.40 per cent., while

of thirty observations on splenectomized animals, in all but two it began in solutions of 0.38 to 0.30 per cent. The resistance of the red cells was found to increase gradually and to reach its maximum at the end of about two months; after further lapse of time up to two years and four months there was no tendency to return to normal. Pel makes a general statement concerning the influence of the serum, to the effect that the serum of a splenectomized dog added to the red cells of a normal dog does not increase the resistance of the latter to hypotonic salt solution, and, *vice versa*, that the addition of normal serum to the red cells of a splenectomized dog does not decrease their resistance. Blood counts showed a slight decrease in the number of red cells after splenectomy, but not enough, in the opinion of Pel, in view also of only slight changes in the percentage of haemoglobin, to indicate a relation to the increased resistance of the red cells. As for the factors responsible for the increased resistance, Pel offers no explanation.

Very recently Gates¹³⁷ has demonstrated that the red blood-cells of splenectomized animals are more resistant than are those of normal animals when submitted to the mechanical damage of long-continued shaking.

The increased resistance of splenectomized animals to blood poisons we observed early in our work with haemolytic serum,²²⁵ and as a result of occasional tests with hypotonic solutions of sodium chloride we reached the tentative conclusion that it was due, in part at least, to increased resistance of the red cells. This supposition we confirmed in a special study,²⁰⁹ in which the cells were tested not only against various strengths of salt solution but by the accurate methods of immunology against specific haemolytic

serum, with also investigation of possible antihæmolytic action of the serum and changes in complement content.

In these tests six dogs were used. One had been splenectomized ten days, a second thirty days, and a third four months before. As the last animal had been given a specific hæmolytic immune serum two months before, thus introducing a new factor, the observations on it were controlled by a fourth dog not splenectomized, which had been given hæmolytic serum five weeks before. Two normal dogs were used as general controls.

TESTS WITH VARYING GRADES OF HYPOTONIC SALT SOLUTION

Chemically pure sodium chloride was dried for two hours at 170° C. and immediately weighed in amounts necessary to make 500 cubic centimetre volumes of salt solution, ranging from 0.1 to 0.5 per cent. in steps of 0.025 per cent. In order to be sure of approximately the same volume of corpuscles in the anæmic as in the normal bloods, the gently defibrinated blood was centrifuged and the serum drawn off. One-tenth of a cubic centimetre of the corpuscular mass was measured accurately in a graduated pipette and placed in three cubic centimetres of each of the various salt solutions. Standard colorimetric scales for comparison were made by laking red cells with distilled water; thus the laking of 0.4 of a cubic centimetre of the corpuscular mass in twelve cubic centimetres of distilled water represented a standard of 100 per cent. hæmolysis. Dilutions of this solution were made so as to have tubes showing the color values of 80, 60, 40 and 20 per cent. hæmolysis. Less than 20 per cent. hæmolysis was considered as a trace of hæmolysis. In most instances this

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TABLE XII
INFLUENCE OF SPLENECTOMY ON THE RESISTANCE OF RED CELLS TO HYDROTOMATIC SALT SOLUTION

Corpuscles of	Percentage strength of salt solu-	Red Cells to Hypotonic Salt Solution										
		0.275	0.300	0.325	0.350	0.375	0.400	0.425	0.450	0.475	0.500	0.525
Dog 30. Normal.....	100	100	100	100	100	100	100	100	60	40	Trace	0
Dog 53. Normal.....	100	100	100	100	100	100	80	40	20	Trace	0	
Dog 51. Splenectomy,												
May 31.....	100	100—	100—	80	60	20	Trace	Trace	0	0	0	
Dog 46. Splenectomy,												
May 10.....	100	100	100—	100—	80	60	40	20	Trace	0	0	
Dog 24. Splenectomy,												
Feb. 10.												
Immune serum, April 16.....	100	100—	80	40	20	Trace	Trace	0	0	0	0	
Dog 43. Immune serum, April 21.....	100	100	100	80	60	20	Trace	0	0	0	0	

scale was entirely satisfactory, but occasionally, although a tube showed 100 per cent. haemolysis colorimetrically, there was, on shaking, a slight macroscopic sediment of incompletely haemolyzed corpuscles; this result was indicated by a minus sign after the approximate percentage of haemolysis. Upon adding the corpuscles to the salt solution, a preliminary reading was made and the mixtures were placed in the refrigerator. The final readings were made at the end of eighteen hours. The results are shown in Table XII.

It will be seen that the blood of the normal dogs (30 and 53) shows haemolysis in fairly high percentages of salt solution, but that the resistance, both maximum and minimum, is increased in all the splenectomized animals. It is true that dog 46, a splenectomized animal, shows initial haemolysis in the same percentage of salt solution as normal dog 53, but inspection will show that, whereas in the normal dog haemolysis is complete at 0.350 per cent., it is not complete in dog 46 until 0.300 per cent. is reached. There can be no doubt that the cells of dog 24, the animal which had been longest splenectomized, show the greatest degree of resistance. That this resistance is due for the most part, if not entirely, to splenectomy is, in view of results with bloods 30 and 53, most probable. On the other hand, it is evident, as shown by the experience with blood 43, that in a non-splenectomized animal the administration of a haemolytic immune serum is capable of causing, after a considerable interval, an increased resistance of the red cells.

TESTS WITH HÆMOLYTIC IMMUNE SERUM

In order to determine the resistance of the corpuscles to a specific hæmolytic immune serum, the following technique was employed: The corpuscles were washed three times in 0.85 per cent. salt solution, and blood suspensions were made of 5 per cent. of red cells as contained in the centrifuged corpuscular mass. The latter rather than whole blood was used because the use of whole blood would be fallacious in the case of anæmic animals. The immune serum was titrated against normal corpuscles, guinea-pig complement was used in doses of 0.1 of a cubic centimetre, and the experiment arranged as indicated in Table XIII.

Technical limitations prevent, in this experiment, as close an estimation of resistance as is possible with hypotonic salt solution, but it can be seen readily that, whereas dilutions of 1/20 and 1/50 produce complete hæmolysis of normal corpuscles, the corpuscles of the abnormal animals were resistant to these dilutions. That the corpuscles of dog 24, which had been splenectomized four months previously, were most resistant is shown by the fact that whereas partial hæmolysis appeared in all other corpuscles in dilutions of 1/250 and 1/300, the corpuscles of this dog completely resisted hæmolysis at such dilutions. The results with the blood of dog 43 demonstrate again that the administration of a hæmolytic serum increases the resistance of the red cells, irrespective of splenectomy.

The results with washed corpuscles in these experiments would indicate that the increase in resistance is a property of the corpuscles themselves, and not the result of antihæmolytic power of the serum, but, to prove this

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TABLE XIII
INCREASED RESISTANCE OF RED CELLS TO HEMOLYTIC IMMUNE SERUM AFTER SPLENECTOMY

	Dilutions of immune serum (June 14, 1912)	1/20	1/50	1/100	1/150	1/200	1/250	1/300	Comple- ment control	Aniso- cytropor control	Blood control
Dog 30.	Normal.....	C.H.*	C.H.	H.	H.	P.H.	P.H.	P.H.	0	0	0
Dog 53.	Normal.....	C.H.	C.H.	H.	H.	P.H.	P.H.	P.H.	0	0	0
Dog 51.	Splenectomy, May 31.....	P.H.	P.H.	P.H.	P.H.	P.H.	P.H.	P.H.	0	0	0
Dog 46.	Splenectomy, May 10.....	P.H.	P.H.	P.H.	P.H.	P.H.	P.H.	P.H.	0	0	0
Dog 24.	Splenectomy, Feb. 10.										
	Immune serum, April 16.....	P.H.	P.H.	P.H.	P.H.	0		0	0	0	0
Dog 43.	Immune serum, April 21.....	P.H.	P.H.	P.H.	P.H.	P.H.	P.H.	P.H.	0	0	0

Washed corpuscle suspensions of

* C. H. = complete haemolysis; H = haemolysis; P. H. = partial haemolysis.

absolutely, other tests were made. These showed conclusively that the increased resistance in splenectomized animals is not due to changes in complement value or increased antihæmolytic power of the serum. The latter point is brought out in the following table, in connection with which it must be pointed out that, as shown by Karsner and Pearce²⁰⁹ in another place, normal dog serum has an antihæmolytic property in a homologous hæmolytic system. This, however, is not increased in the splenectomized animal.

It is seen that in all the dilutions of dog serum used the action of one dose of amboceptor and of complement was hindered, but in none of the dilutions was the anti-hæmolytic property sufficient to hinder the action of two doses of complement and of amboceptor. Weaker dilutions were not considered necessary, because it seems certain that between the action of a dilution of 1/64 on one dose of hæmolysin and of whole serum on two doses no fine gradation could exist.

TESTS WITH SAPONIN

In a third series of experiments we have investigated the resistance of the red cells to saponin. This was done because of the objection raised by McNeil²⁶⁴ that resistance tests with hypotonic salt solution give results different from those obtained when the washed cells are tested with saponin. By immersing the cells in hypotonic salt solution, thereby introducing a complicating factor, he found that their subsequent resistance to hypotonic solutions was changed, but not their resistance to saponin. He concludes that saponin tests the resistance of the cell

TABLE XIV
TESTS SHOWING ABSENCE OF INCREASED ANTI-HAEMOLYTIC POWER IN SERUM OF SPLENECTOMIZED DOGS

envelope and hypotonic salt solution tests the concentration of salts inside the cell, a condition that, according to him, is not affected in disease.

Our methods have been as follows:

Saponin (Merck's Saponin Purum) was prepared in normal salt solution (0.85 per cent.) in strengths (varying by 0.0025 gm.) from 0.005 to 0.030 gms. per litre. As all the tests were performed and completed during a period of a few days, the factor of deterioration of the solution was avoided. In every case blood was drawn from a vein by means of a syringe and the red cells washed three times in normal salt solution. One-tenth cubic centimetre of a 50 per cent. suspension of such cells was added to 11 tubes of graded strengths of saponin solution and the amount in each made up to 2 c.c. with normal salt solution. To a parallel series of 11 tubes of hypotonic salt solution varying in concentration from 0.25 per cent. NaCl to 0.55 per cent. NaCl equal amounts of the washed red blood-cells were added, and both saponin and salt solution series were incubated for two hours at 37.5° C., with occasional gentle agitation. At the end of two hours the different degrees of haemolysis were determined. In each series several tubes in the middle showed partial haemolysis, with complete haemolysis at one extreme and none at the other; so that accurate comparison could be made without resorting to the enumerative method employed by McNeil.

By this method it was found that the red cells of splenectomized animals exhibit the same increase in resistance to saponin haemolysis that was observed in the experiments with hypotonic salt solutions and haemolytic immune serum. Comparative tests using hypotonic salt solution showed

that the changes, both in maximal and minimal resistance, were the same as were obtained with saponin.

These studies on the resistance of red blood-cells confirm the results of others who have used graded hypotonic salt solutions as a means of measuring the resistance of red cells and add new facts concerning the degree of resistance to specific haemolytic immune serum and to saponin. The second group of experiments indicates, as far as it is possible, by immunological methods, that the increased resistance is a property of the red cells themselves, and not the result of increased antihæmolytic power of the serum.

Since these observations were made, Kolmer²²² has demonstrated that the erythrocytes of splenectomized dogs show an increased resistance also to haemolysis by cobra venom. This increased resistance was observed as early as four days after splenectomy and usually persisted for a period of about three weeks, when the resistance gradually decreased to the normal or slightly beyond. His control experiments show, moreover, that the temporary character of this increase in resistance is peculiar to venom and differs from the increased resistance to hypotonic salt solutions which persists for long periods of time. The increased resistance to hypotonic salt was constantly present in all the dogs he tested.

To what is this increased resistance due? That it is a concomitant of the anaemia following splenectomy and occurs also in many anaemias without splenectomy is generally admitted. It is but natural, therefore, that some investigators should ascribe the increased resistance to the presence in the blood of a large number of newly-formed red blood-cells, generally supposed to be more resistant

than the mature forms. This view, as we have seen, was first put forth by Pugliese and Luzzatti (1900) and has considerable support. Our own experience leads us to believe that the increased resistance is closely related to the anaemia, or to the process of repair that accompanies the anaemia; but, on the other hand, we have not found that the anaemia of splenectomy is characterized by the presence in the blood of cells of immature type as nucleated cells, or by an appreciable increase in the so-called reticulated or skeined cell, which are now considered as recently formed of young cells.

We have examined many animals for these cells at periods varying from four days to one year after splenectomy,³³⁹ but have failed, as a rule, to find an appreciable increase, and never more than 2 per cent. Moreover, Pepper and Peet³⁴³ have found that in experimental anaemia (in the rabbit) due to phenylhydrazine these cells are no more resistant to haemolysis by hypotonic salt solution than are normal red cells. It has therefore been impossible for us to bring forth definite evidence that this increased resistance of the cells after splenectomy is due to the presence of young forms. On the other hand, in view of the fact that this phenomenon is present in anaemia in animals with intact spleen, we are not prepared to eliminate anaemia entirely as a factor. Banti,²⁹ however, denies the influence of anaemia. He argues that (1) the degree of increase in resistance is not proportional to the anaemia, (2) it may develop in the absence of anaemia, (3) it may persist for many years, and (4) that red cells in the splenic vein are less resistant than those in the general circulation. He also makes the point (5) that after the injection of haemolytic serum, in spite of the marked

anæmia and the many young cells in the circulation, the resistance to this poison is decreased, not increased.

Our opinion on these points is as follows:

1. Although we believe that the increased resistance is closely associated with the anæmia and may probably be due to some factor accompanying it or the initial regenerative processes in the blood, we have not committed ourselves absolutely to this view, as Banti seems to think we have, for the reason that we have never been able to obtain satisfactory proof on this point. We do not, however, consider Banti's objection to be very substantial, for, although it is quite true that the degree of anæmia arising in a splenectomized animal is not always proportional to the increase in resistance, there is no reason to assume that there should be a proportional relation.

2. Banti's observations concerning increased resistance in the absence of anæmia are not conclusive. We have never failed to find a decrease of red cells and hæmoglobin at some time after splenectomy; this may come early or may be late, but in our experience it never fails, and, though as a rule long continued, may be slight and evanescent. It may, however, easily be missed if continued counts are not made at frequent intervals for long periods of time. In Banti's experiments the four animals that failed to develop anæmia, but did show increased resistance, were examined on (1) the second and twenty-fourth, (2) the third and thirteenth, (3) the third and fifteenth, and (4) the fifth and twenty-seventh days, respectively. Our experience has shown that in dogs on special diets the change in the blood may be long delayed or may be present in slight evanescent form in the interval periods. On the other hand, in some dogs we have found occasion-

ally a slight increase of resistance before the frank fall in the number of red cells, but, as it coincided with or was quickly followed by a decrease in haemoglobin, we could not rule out the influence of the factors causing anaemia.

3. So, also, the observation concerning increased resistance in man eight years after splenectomy is inconclusive, for Banti's blood examination shows 4,950,000 red cells and 70 per cent. haemoglobin, a figure for haemoglobin, of doubtful interpretation. Much more work, both experimental and clinical, must be done before we can reach a definite conclusion concerning the points here discussed.

4. The observations concerning the lessened resistance of cells in the splenic vein are offered in support of Banti's theory* of the spleen as an organ concerned in haemolysis. While we believe the spleen is concerned in the destruction of red cells, our observations do not confirm the experiments put forth by Banti to prove the decreased resistance of cells of the splenic vein blood. This will be discussed elsewhere,† as will also, in that it brings in a new factor, the matter of (5) decreased resistance of red cells immediately after the injection of a haemolytic serum.

Although we offer these statements to support the possibility of an association between anaemia and increased resistance, we do so without presenting definite proof. Moreover, we do not base this support, though Banti seems to think we do, on the presence of young cells in the blood.

* Based on Bottazzi's theory that the spleen has special (haemocatatonistic) action upon red blood-cells as they pass through the organ and as a result of which they became less resistant. The absence of the spleen, according to this theory, does away with this action, and the red cells therefore are more resistant in splenectomized animals.

† See page 87.

The skeined cells we have shown are no more resistant than are the mature cells. We do think, however, that it is some factor intimately associated with the causation or repair of the anæmia following splenectomy, and not the mere absence of the spleen, that is responsible for the increased resistance, and that this factor is operative in other anæmias, in the presence of the spleen. That this factor may be entirely independent of the anæmia we willingly admit.

III. LESSENED TENDENCY OF HÆMOLYTIC AGENTS TO CAUSE HÆMOGLOBINURIA AND JAUNDICE AFTER SPLENECTOMY

In the preceding section we have presented the evidence concerning the increased resistance of the erythrocytes as determined by the behavior of these cells to various lytic agents. In the present section we offer the evidence concerning the closely related phenomenon—the lessened tendency to hæmoglobinuria and icterus—exhibited by splenectomized dogs. To establish this point we have examined the urine for hæmoglobin and bile-pigment^{335, 339} and determined the changes in the blood as shown by red-cell counts and hæmoglobin estimations.³³⁶ The hæmolytic agent used in every instance has been hæmolytic immune serum.*

Female dogs were used almost exclusively in order that the tests for hæmoglobin and bile might be made on urine obtained by catheterization. All operations were under ether anæsthesia, as were also the injections of serum.

* Rabbits were injected five times with five to ten cubic centimetres of dog's blood at intervals of five to seven days and bled about one week after the last injection.

The injections were either into a small vein of the leg or into the jugular vein. Each experiment on a splenectomized animal was controlled by the injection of a normal animal with the same serum. When the animals were of approximately the same weight they received the same amounts of serum; but when the weight varied more than half a kilo they received, with a few exceptions, corresponding doses per kilo. of body weight.

The urine of all animals was examined for coagulable protein, haemoglobin, and bile pigment. For the diagnosis of jaundice, the appearance of bile-pigment in the urine has been deemed sufficient. Table XV shows the effect of a weak haemolytic serum, administered three days after splenectomy.

TABLE XV

EFFECT OF HÆMOLYTIC SERUM ADMINISTERED THREE DAYS AFTER SPLENECTOMY, WITH CONTROL

Date	Dog 11. Weight 10,000 gm.	Dog 13. Weight 8,990 gm.
Dec. 11, 1911	Splenectomy	Control
Dec. 14, 1911	Urine normal; 2.5 c.c. haemolytic serum in vein	Urine normal; 2.5 c.c. haemolytic serum in vein
Dec. 15, 1911	Urine normal	Bile test positive; no haemoglobin, no albumin
Dec. 16, 1911	Urine normal; 4.5 c.c. of same serum in vein	Bile test positive; no haemoglobin, no albumin; 4.5 c.c. of same serum in vein
Dec. 17, 1911	Trace of albumin; no bile, no haemoglobin	Bile test positive; albumin present, no haemoglobin
Dec. 18, 1911	Trace of albumin; no bile, no haemoglobin	Bile test positive; albumin present, no haemoglobin
Dec. 19, 1911	Trace of albumin; no bile, no haemoglobin Ligation of common bile duct under ether anaesthesia	Bile test positive; albumin present, no haemoglobin Died, under ether, during operation to remove spleen
Dec. 20, 1911	Bile-pigments in urine	
Dec. 21-22, 1911	Bile-pigments in urine increasing	
Dec. 23, 1911	Bile-pigments in urine increasing Killed by chloroform	

In this experiment the haemolytic serum was not powerful enough to cause a destruction of blood of sufficient grade to produce haemoglobinuria, although it did cause in the control animal enough destruction to produce jaundice; on the other hand, the splenectomized animal was free from jaundice.

The objection might be raised, in connection with this experiment, that the jaundice of the control animal might be due to the fact that, as the smaller of the two animals, it received a relatively larger dose of serum. This objection is not tenable, as is shown by Table XVI. In the experiment here presented, a stronger serum was used and the amount injected was adjusted to the weight of the animals.

TABLE XVI

EFFECT OF HÆMOLYTIC SERUM SIX DAYS AFTER SPLENECTOMY, WITH
CONTROL

Date	Dog 3. Weight 6,400 gm.	Dog 7. Weight 10,580 gm.
Dec. 14, 1911	Splenectomy	Control
Dec. 20, 1911	Urine normal	Urine normal
Dec. 21, 1911	0.5 c.c. serum per kilo.	0.5 c.c. serum per kilo.
Dec. 22, 1911	Hæmoglobinuria; no bile-pigment	Hæmoglobinuria; marked jaundice
Dec. 23, 1911	Hæmoglobinuria; no bile-pigment	Hæmoglobinuria; marked jaundice
Dec. 24, 1911	Trace of hæmoglobinuria; no bile-pigment	No hæmoglobinuria; marked jaundice
Dec. 25, 1911	No hæmoglobinuria; no bile-pigment	No hæmoglobinuria; marked jaundice
Dec. 26-27, 1911	Faint trace of bile-pigment	Much bile-pigment in urine
Dec. 28, 1911	No bile-pigment	Much bile-pigment in urine
Dec. 29, 1911	No bile-pigment	Much bile-pigment in urine

That the same results are obtained after longer periods of time have elapsed is shown in Table XVII, which presents the results obtained sixty-five days after splenectomy.

TABLE XVII
DECREASED TENDENCY TO JAUNDICE SIXTY-FIVE DAYS AFTER SPLENECTOMY

Date	Dog 10. Weight 9,720 gm.	Dog 22. Weight 6,710 gm.
Dec. 9, 1911	Splenectomy	Control
Feb. 12, 1912	Urine normal	Urine normal
Feb. 13, 1912	0.25 c.c. serum per kilo. into vein	0.25 c.c. serum per kilo. into vein
Feb. 14, 1912	Urine free from hæmoglobin and bile-pigment	No hæmoglobinuria; bile-pigments present
	1 c.c. same serum per kilo. into vein	1 c.c. per kilo. of same serum into vein
Feb. 15, 1912	No hæmoglobinuria; no bile-pigment	Marked hæmoglobinuria; much bile-pigment
Feb. 16, 1912	No hæmoglobinuria; no bile-pigment	No hæmoglobinuria; much bile-pigment
	2 c.c. per kilo. of another serum into vein	No third injection. Spleen excised
Feb. 17, 1912	Hæmoglobinuria; faint trace of bile-pigment	Well marked bile reaction
Feb. 18, 1912	No hæmoglobinuria; faint trace of bile (?)	Well marked bile reaction
Feb. 19, 1912	No hæmoglobinuria; faint trace of bile (?)	Well marked bile reaction
Feb. 20-21, 1912	Common bile-duct ligated Large amount of bile-pigment in the urine Chloroformed	Well marked bile reaction Chloroformed

In all of these experiments the splenectomized dogs show less tendency to jaundice and usually to hæmoglobinuria than do the normal dogs with corresponding doses of the same hæmolytic serum, and this, as will be shown later, we have found to be characteristic of animals that have been splenectomized for various periods up to one year. Beyond that period we have made no observations.

CHAPTER III

CONCERNING THE SUPPOSED REGULATORY INFLUENCE OF THE SPLEEN IN BLOOD DESTRUCTION AND REGENERATION

- A. IN RELATION TO THE DECREASED TENDENCY
TO HÆMOGLOBINURIA AND JAUNDICE: (1) THE
RELATION OF SPLEEN TO THE LIVER AND THE
FORMATION OF BILE FROM HÆMOGLOBIN. (2)
THE INFLUENCE OF THE COURSE OF THE BLOOD
TO THE LIVER. (3) THE INFLUENCE OF ANÆMIA.
(4) THE INFLUENCE OF THE INCREASED RESIST-
ANCE OF THE RED CELLS. (5) ARE SPLENIC EX-
TRACTS HÆMOLYTIC? (6) POSSIBLE INFLUENCE
OF FATTY ACIDS AND LIPOIDS.

In the preceding chapters have been presented the three most important phenomena—anæmia, increased resistance of the erythrocytes, and decreased tendency to jaundice—which follow splenectomy. A discussion of these involves a presentation of the literature and of experiments dealing with the supposed regulatory influence of the spleen. As the increased resistance of the red cells has a relation both to the problem of anæmia and to that of the decreased tendency to jaundice, it will not be discussed separately, but in relation to each of these.

How does the absence of the spleen influence the occurrence of hæmoglobinuria and jaundice?

(1) One improbable explanation, that the absence of the spleen prevents the secretion of bile by the liver, may be dismissed immediately, for, as shown in Tables XV and XVII, the ligation of the bile-duct in the splenectomized

animal³³⁵ causes the appearance of bile in the urine within twenty-four hours. On the other hand (Table XVII), the excision of the spleen in an animal with haemolytic jaundice does not immediately lessen the elimination of bile through the urine.

Does the spleen take part in the formation of bile-pigment from haemoglobin? As the solution of this problem necessitated the study of haemoglobinæmia in normal animals, we undertook an extensive investigation³³⁴ under the following heads: (1) The degree of haemoglobinæmia necessary in order to recognize free haemoglobin in the serum; (2) the degree of haemoglobinæmia necessary for the escape of haemoglobin through the kidneys; (3) the percentage of haemoglobin eliminated by the kidneys; (4) the degree of retention of haemoglobin necessary to cause jaundice; (5) the influence of the absence of the spleen on the elimination or retention of haemoglobin and the occurrence of jaundice.

METHODS

Defibrinated dog blood was haemolyzed with distilled water, sodium chloride was added to render the solution isotonic with dog blood, the haemolyzed blood was centrifuged to remove the stroma, and the haemoglobin content was then determined with a Fleischl haemoglobinometer. Definite amounts of the haemoglobin solution, always freshly prepared, were allowed to flow from a burette into a small branch of the femoral vein. The first appearance of haemoglobin in the urine was determined by a catheter in the bladder or by a catheter in one ureter. In order to aid the flow of urine, each dog received 300 cubic centimetres of water by stomach-tube. From time to time the skin was punctured and blood was drawn into capillary

tubes to determine how early free hæmoglobin appeared in the serum.

The elimination of hæmoglobin in the urine was estimated by rendering the urine acid with hydrochloric acid to about N/10 and comparing this solution of acid hæmatin, suitably diluted, with a 1 per cent. solution made according to the Sahli method from blood containing 100 per cent. of hæmoglobin by the Fleischl scale. A Dubosc colorimeter was used for making the comparison.

The quantities of hæmoglobin are designated in the table (Table XVIII) in grammes, calculated on the assumption, for the sake of convenience, that blood giving a reading of 100 per cent. by the Fleischl scale contains 14 per cent. of hæmoglobin. This figure is, of course, only approximately correct, but, as only relative quantities are of importance in this work, an approximate determination of the absolute quantities of hæmoglobin is sufficient.

In order to determine quantitatively the amount of hæmoglobin which must be retained in order to cause jaundice, decreasing amounts of hæmolyzed blood were injected intravenously into a series of normal dogs; in each case the percentage elimination by the kidney and occurrence or non-occurrence of bile-pigment in the urine were noted.

The results of these experiments are shown in Experiments I to IX in Table XVIII.

It is seen (Experiments II, VI, VII, VIII, IX) that the retention of 0.39 gramme of hæmoglobin per kilo. caused marked choloria; of 0.23 gramme, slight choloria for twenty-four hours; and of 0.22 gramme, a very faint choloria for eight hours; the retention of 0.18 and 0.19 gramme per kilo. of body weight failed to cause choloria.

The percentage of hæmoglobin eliminated by the kid-

TABLE XVIII
THE ELIMINATION AND RETENTION OF HEMOGLOBIN IN NORMAL AND SPLENECTOMIZED Dogs

ney appears to be a variable quantity. Thus, in Experiment VI, 32.5 per cent. of the hæmoglobin injected was eliminated by the kidney; in Experiment VII, 26.8 per cent.; in Experiment VIII, 17.1 per cent.; in Experiment IX, 35.9 per cent.

In these four experiments the hæmoglobin solution was rapidly injected during a period of from four to thirteen minutes. When the solution was introduced more slowly a much larger amount could apparently be cared for in the liver without the production of jaundice. Thus, if we refer again to Table XVIII, Experiment I, in which the solution was introduced at intervals throughout a period of fifty-six minutes, we find that an amount of hæmoglobin was retained equal to 0.33 grammes per kilo., without bile-pigments occurring in the urine.

These experiments seem definitely to establish the mechanism by which free hæmoglobin is removed from the blood-serum under normal conditions. Our conception of this mechanism is as follows: The kidney does not eliminate hæmoglobin until its concentration in the blood-serum reaches a certain level. This concentration, we conclude from Experiments I, II, III, is about that produced by the presence of 0.06 grammes of free hæmoglobin per kilo. of body weight. As soon as the concentration of the hæmoglobin in the serum is above this point, the hæmoglobin passes through the kidneys and we have hæmoglobinuria, but as soon as it falls below this amount, the hæmoglobinuria ceases. However, other tissues, of which presumably the liver is the most important, appear to take up hæmoglobin as soon as mere traces are present in the serum, and continue to remove it from the serum, whether the renal threshold is exceeded or not. Therefore, whenever

the kidney is removing haemoglobin from the serum, these other tissues are also removing it. Under the conditions of these experiments the kidneys removed 17 to 36 per cent., and the liver (and other tissues?) 64 to 83 per cent.

The haemoglobin which the liver removes is changed into bile-pigment, which, if it is not produced in too large amounts, or if the haemoglobin is not taken to the liver too rapidly, passes out as bile-pigment in the usual manner through the bile-passages. On the other hand, if the haemoglobin is taken up by the liver in larger quantities, and especially if this occurs rapidly, the bile-pigment is formed faster than the bile capillaries can remove it, and it is reabsorbed into the circulation and appears in the urine.

The effect of splenectomy on this process was determined in part by observations on the same animal before and after splenectomy, and in part on animals splenectomized for various lengths of time (Table XVIII, Experiments X to XV).

These six experiments on splenectomized animals, in all of which bile-pigments appeared in the urine for a short time and in small quantities after the retention of 0.44, 0.31, 0.25, 0.28, 0.26, and 0.22 gm. per kilo., respectively, indicate that the threshold for jaundice in splenectomized dogs is approximately 0.22 grammme per kilo., the same as in the experiments (VI to IX) with normal dogs, in which the threshold was found to be between 0.18 and 0.22 grammme per kilo.

When we examine the percentage of haemoglobin eliminated by the kidneys in the six splenectomized animals, we find that it runs a trifle lower than the limits determined for normal animals, being 18.8, 16.8, 21.1, 27.5, 7.2 (?), and 18.4 per cent. (average, excluding the fifth figure,

20.5 per cent.), as compared with 32.5, 26.8, 17.1, 35.9, 16, and 26.5 per cent. (Experiments VI to XI), with an average of 25.8 per cent. This difference is, however, so slight that we can conclude that splenectomy has no influence in increasing the elimination of free haemoglobin by the kidneys, nor does it, as is shown by the occurrence of choluria in each of the experiments, influence the ability of the liver to form bile-pigments from haemoglobin, or interfere with the elimination of these pigments. Thus one of the possible explanations for the failure of jaundice to follow the administration of a haemolytic serum in splenectomized animals is shown to be untenable.

(2) THE INFLUENCE OF THE COURSE OF THE BLOOD TO THE LIVER

Although the experiments described in the preceding section led to negative conclusions, there is another possible factor, a purely mechanical one; that is, the relation of the spleen to the blood supply of the liver. Inasmuch as the spleen is undoubtedly a seat of destruction of red cells, the splenic blood must carry to the liver haemoglobin in larger amounts, or more concentrated, than does the blood reaching the liver through the hepatic artery in the absence of the spleen. This difference in haemoglobin content, under the conditions mentioned, might be sufficient to explain, in a purely mechanical way, the lessened tendency to jaundice after removal of the spleen. This possibility was investigated in two groups of experiments: in one²³ haemoglobin was injected in the general circulation (femoral vein) and into portal circulation (mesenteric vein), and the influence of these two procedures on the occurrence of haemoglobinuria and jaundice was studied. In another²²

set of experiments the blood from the spleen was diverted from the liver by ligation of the splenic vein, as well as by an Eck fistula or an anastomosis of the splenic vein with the vena cava, and the occurrence, under these conditions, of jaundice due to the administration of haemolytic agents was studied.

In connection with this problem it may be recalled that Ponfick²⁵⁸ applied the term "spogenous" (*σπόδος* waste products) to the spleen of haemolysis, swollen in consequence of the accumulation of disintegrating erythrocytes, and that he noted that simultaneously the liver eliminates a bile very rich in pigment, and suggested that this latter is derived from the haemoglobin set free in the spleen, carried by the portal circulation to the liver, and removed by this organ. Ponfick further expressed the view, based on experiments not quoted in detail, that the liver could completely remove and transform into bile-pigment liberated haemoglobin up to the extent of one-sixtieth of the total haemoglobin of the body, but that haemoglobin set free in excess of this amount passes through the liver and is eliminated by the kidneys, causing haemoglobinuria. One-sixtieth of the total haemoglobin in the dog is about 0.18 gm. per kilo. In the preceding section we have shown that the injection of 0.14 to 0.35 gm. per kilo. of haemoglobin as laked blood will cause the appearance of haemoglobinuria, but that a factor of great importance, apparently overlooked by Ponfick, is the rate at which the haemoglobin is liberated in the circulation. The more slowly it is introduced the larger is the quantity that the liver can take up without permitting the concentration in the blood to reach at any time that required for the production of haemoglobinuria. Also, we have shown that, while small

amounts of injected hæmoglobin are removed by the liver and presumably excreted as bile-pigments in the bile without the occurrence of jaundice, if the injected hæmoglobin be in excess of 0.30 to 0.40 gm. per kilo. the liver is unable to eliminate all the bile-pigment formed from the excess of hæmoglobin, and some of the bile-pigment is reabsorbed from the liver and under these circumstances appears in the urine. It was noted in this respect, also, that the rate of injection is of greater importance in determining the amount of hæmoglobin that the liver will tolerate without the appearance of bile-pigments in the urine. Very slow but long-continued administration of hæmoglobin can eventually overtax the hepatic excretory power and lead to the appearance of bile-pigments in the urine, although the hæmoglobin transformation may have been slow enough to permit of its continued adequate removal from the circulation by the liver with at no time the development of hæmoglobinuria. Thus the first effect of hæmoglobin liberation into the blood is an increased bile pigment content of the bile. This was shown experimentally by Tarchanoff.⁴²⁶ If the amount of hæmoglobin be small enough and its liberation slow enough, this is the only effect. A slightly larger amount, rapidly liberated, will produce hæmoglobinuria. A still larger amount, extremely slowly liberated, will produce bile in the urine. An equal amount liberated at an intermediate rate may produce both hæmoglobinuria and bile-pigments in the urine.

Following Ponfick, many other workers have attributed importance to the spleen as the site of disintegration of erythrocytes; among these may be mentioned Hunter,¹⁸⁹ Gabbi,¹³⁵ and Mya.³¹² Bottazzi,⁵³ in his studies of the blood after splenectomy, noted an increased resistance of

the erythrocytes to hypotonic salt solutions, and it was to this factor that Banti²⁸ attributed the greater resistance and diminished tendency to jaundice of splenectomized animals receiving haemolytic agents.

However, Pugliese and Luzzatti³⁶⁴ did not agree with Bottazzi, but, noting, with Banti and others, the diminished tendency to jaundice after splenectomy, they made further studies along the lines suggested by Ponfick's observations and elaborated the following hypothesis: The spleen is the natural location for the disintegration of erythrocytes after the administration of haemolytic poisons, and the haemoglobin so liberated is carried directly by the portal system to the liver, there to be converted into bile-pigment and to be excreted in the bile, or, if present in great quantity, to be reabsorbed and appear in the urine and tissues as bile-pigments and thus produce jaundice. On the other hand, in the absence of the spleen, the blood-cells undergo disintegration elsewhere, probably chiefly in the bone-marrow, as suggested by Martinotti and Barbacci.²⁷⁷ Haemoglobin liberated in the bone-marrow could, under these circumstances, reach the liver only through the general circulation. It would therefore be diluted and, moreover, would reach the liver largely through the hepatic artery—a vessel normally carrying blood for nutritive purposes, not for purposes of elaboration. For these reasons it is to be expected that, in the splenectomized animal, the haemoglobin would reach the liver much more gradually and at a rate, indeed, which might well lie within the capacity of the liver for complete excretion as bile-pigment, and hence no reabsorption of bile-pigments would occur and jaundice would not develop.

Pugliese and Luzzatti were able to show by the aid

of a bile fistula that, while the other constituents of the bile are but little altered by splenectomy, the bile-pigments are reduced to about one-half. Moreover, while the bile of the splenectomized animal shows an increase in the bile-pigments after the administration of haemolytic poisons, this is not so pronounced as in the normal animal; the increased pigmentation of the bile is, however, of longer duration in the splenectomized animal.

In order to test the hypothesis of Pugliese and Luzzatti, we have injected²³ a constant amount per kilo. of haemoglobin solution in the form of laked blood into a series of dogs, injecting each at least twice—once into the general circulation by way of the femoral vein and once into the portal circulation by way of a mesenteric vein. The rate of injection has been always the same. In some instances the femoral injection was given first, in other instances the mesenteric. At least five days were allowed to elapse between injections. We have thus been able to study the effect of the site of the injection upon the development of haemoglobinuria and of bile-pigments in the urine, and, from our results, believe we may draw conclusions as to the fate of haemoglobin when liberated into the portal system, on the one hand, or into the general circulation, on the other.

In these experiments a fasting normal dog was bled, the blood defibrinated, the cells thrown down by a centrifuge, and the supernatant serum removed. About four volumes of distilled water were then added to the cells to induce haemolysis, and the mixture agitated for fifteen to twenty minutes. The solution was then centrifugalized rapidly for twenty minutes to remove the cell stromata, was made isotonic by addition of sodium chloride, and cen-

trifugalized to remove any globulin thrown out of solution upon adding the salt. One cubic centimetre of this solution was then diluted with 99 c.c. of distilled water and its haemoglobin strength determined in a Fleischl-Miescher haemoglobinometer. Into a normal dog was then injected intravenously as much of this solution as should equal either 0.3 gm. or 0.4 gm. of haemoglobin per kilo. of body weight. Injections were given at such a rate that the entire injection should occupy one minute per kilo. of body weight. All bleedings and injections were made under ether anaesthesia. Injections into the general circulation were made into one of the small veins of the leg. Portal injections were made by drawing a loop of intestine from the abdomen under aseptic precautions and injecting through a needle into a small mesenteric vein. In some instances water was given by stomach-tube at the close of the operation. The urine was then collected, the dog being kept in a metabolism cage, and, if haemoglobin appeared, the amount was estimated either directly in the Fleischl-Miescher haemoglobinometer or by comparison with a standardized acid haematin solution. In addition, we followed the course of the jaundice by observing the persistence of bile-pigments in the urine after haemoglobin injection into either the mesenteric or the femoral vein. The urine was examined for bile-pigments by the Rosenbach test.

The results as regards haemoglobinuria are shown in Table XIX. In each of five dogs used, the output of haemoglobin by the kidney was much less when the haemoglobin was introduced into the mesenteric vein than when introduced into the femoral vein, and this is true regardless of which injection was performed first. This we attribute

to the removal of the haemoglobin to a greater extent by the liver when the injection is made into a mesenteric vein, with the result that the haemoglobin reaching the general circulation is less concentrated and is less likely to be eliminated by the kidneys and appear in the urine.

TABLE XIX
INFLUENCE OF SITE OF INJECTION ON AMOUNT OF HÆMOGLOBIN ELIMINATED
IN THE URINE

Hæmoglobin injections	Date	Gm. of hæmoglobin per kilo, eliminated in the urine after injection into	
		Femoral vein	Mesenteric vein
Dog 26 (0.4 gm. per kilo.)	Mar. 20	0.085	0.043 0.029
	Mar. 26		
Dog 12 (0.3 gm. per kilo.)	Feb. 25		None None
	Apr. 15	0.043	
Dog 5 (0.3 gm. per kilo.)	Jan. 9	0.024	Trace None
	Jan. 15		
	Mar. 2		None
	Mar. 26	0.026	
Dog 3 (0.3 gm. per kilo.)	Jan. 7	0.017	Trace None
Splenectomized	Jan. 15		
	Feb. 19		None
	Mar. 2		
	Mar. 20	0.025	0.014 0.010
Dog 49 (0.3 gm. per kilo.)	Feb. 25		
Three months after splenectomy	Apr. 15		

The results of the study of the degree and persistence of jaundice (as indicated by bile-pigments in the urine) in the dogs after the two types of injection are shown in Table XX.

It will be seen that in the six dogs studied, the jaundice was distinctly more persistent after mesenteric than after femoral injection, and this was true regardless of which injection was made first. In Dog 4 two successive injections were made into the femoral vein to determine whether the second injection would give a result notably different from the first. Such was not the case, the duration of

the bile-pigments being the same after each injection when both were made into the femoral vein.

In our studies both of haemoglobinuria and of the persistence of jaundice after haemoglobin injections we have employed splenectomized as well as normal dogs, but have found that the mere absence of the spleen has no influence upon the fate of haemoglobin injected into either the general or portal circulation. The place of injection is the important factor.

TABLE XX

PERSISTENCE OF BILE-PIGMENT IN THE URINE AFTER HÆMOGLOBIN INJECTION AS DETERMINED BY POINT OF INJECTION

Hæmoglobin injections	Date	Persistence after injection into	
		Femoral vein	Mesenteric vein
Dog 4 (0.3 gm. per kilo.)	Jan. 9	days 4	days 4
	Jan. 16	4	
Dog 5 (0.3 gm. per kilo.)	Jan. 9	3	12+
	Jan. 15		
Dog 25 (0.4 gm. per kilo.)	Mar. 21	4	7+
	Mar. 26		
Dog 26 (0.4 gm. per kilo.)	Mar. 20	4	7+
	Mar. 26		
Dog 12 (0.3 gm. per kilo.)	Feb. 25		5
	Apr. 15	4	
Dog 3 (0.3 gm. per kilo.)	Jan. 7	0	9
	Jan. 15		
Splenectomized.....	Feb. 19		4
	Mar. 2		
Dog 49 (0.3 gm. per kilo.)	Mar. 20	0	4
Three months after splenectomy	Feb. 25	4	
	Apr. 15		6

These experiments indicate, therefore, that when haemoglobin is set free in the portal circulation a larger amount is held by the liver and converted rapidly into bile-pigment than is the case when it is set free in the general circulation, and that under the former condition overloading of the liver with bile-pigment more readily occurs and jaundice is more apt to develop.

This mechanical influence must therefore be a factor, though not necessarily the only factor, in the lessened tendency after splenectomy to the jaundice which follows blood destruction due to haemolytic agents, for whether the spleen be an active factor in destroying the erythrocytes or whether it plays merely a passive part as a place for the deposition of the disintegrating cells, there can be no question that in this organ a large number of cells undergo their final disintegration after the action of haemolytic poisons, and that the haemoglobin there liberated passes by the portal system directly to the liver. When the spleen is removed this disintegration occurs in other organs, notably in the lymph-nodes and bone-marrow, and the haemoglobin from these organs passes not into the portal but into the general circulation, from which it reaches the liver more gradually and in a more dilute form. Jaundice is therefore less apt to occur under such circumstances, when a haemolytic agent is administered, than is the case in the normal animal.

Other experiments giving essentially the same result and supporting the injection experiment described above are those* in which, by ligation of the splenic vein and blood-vessel anastomosis, the splenic blood was diverted from the liver and haemolytic agents were then administered.²²⁹ These showed the same decreased tendency to jaundice that is shown by splenectomized animals.

(3) THE INFLUENCE OF ANAEMIA

This we have investigated in some detail.³³⁶ In our earlier work it was noticed more or less accidentally that

* See page 130.

an injection of hæmolytic serum into anæmic dogs, whether splenectomized or not, did not cause jaundice so readily as in dogs with normal red-cell count and normal hæmoglobin content. This observation suggested that it might not be the mere absence of the spleen, but secondary changes in the blood consequent upon the absence of the spleen, that in addition to the mechanical factor prevented the appearance of jaundice in splenectomized animals. We therefore turned our attention to the condition of the blood in all splenectomized animals.

As we have shown in the first chapter, splenectomy is followed by a moderate anæmia in which a decrease of hæmoglobin is especially prominent. The lowest level of this anæmia corresponds to the third to the sixth week, and it occurred to us that, in this early period, the lessened tendency to jaundice might be associated in some way with the coexistent anæmia. Obviously it was possible to test this hypothesis by studying the effect of a hæmolytic serum on dogs rendered anæmic in some other way than by splenectomy. This was done by bleeding, as is shown in an experiment (see Table XXI) in which a normal dog with high hæmoglobin content is contrasted with a dog rendered moderately anæmic by bleeding.

In this experiment the anæmic dog, although it received the same amount of serum, proportionately, and, on account of its greater weight, twice as much, actually, as the control, failed to develop hæmoglobinuria or jaundice, and this in spite of the fact that the actual fall in hæmoglobin and red blood-cells was more rapid and greater than in the control. Anæmia would appear, therefore, to be an important factor in lessening the tendency to jaundice. How the anæmia acts to lessen the jaundice is not indicated by

our experimental data. A possible explanation which may be offered, however, is the following. We have shown that the liver exhibits a saturation point for haemoglobin, so

TABLE XXI
EFFECT OF HÆMOLYTIC SERUM ON A DOG RENDERED ANÆMIC BY BLEEDING.
NORMAL CONTROL

Date	Anæmic dog	Date	Control dog
April 18, 1912	Weight, 10,740 gm. Urine: free of albumin 179 c.c. of blood taken from jugular vein	April 16, 1912	Weight, 5,350 gm. Urine: no albumin, no bile.
April 20, 1912	150 c.c. of blood taken from jugular vein		Blood: red cells, 5,390,- 000; haemoglobin 107 per cent.
April 21, 1912 10 A. M....	Urine: no albumin, no bile-pigment Blood:red cells, 4,450,000; haemoglobin 87 per cent. Fragility: 0.3+; 0.4-	11.45 A. M. 2.45 P. M. 3.15 P. M.	Fragility: 0.4+; 0.5-. Received in vein 0.25 c.c. per kilo. of same serum as anæmic dog. Much bile in urine. Blood: red cells, 5,330,- 000; haemoglobin 98 per cent.
April 21, 1912, 11.10 A. M. 5 P. M.	Received 0.25 c.c. hæmo- lytic serum per kilo. in vein Urine: no albumin, no bile Blood:red cells, 3,400,000; haemoglobin 54 per cent.	6.30 P. M.	Urine: contains haemo- globin and much bile Blood: red cells, 4,660,- 000; haemoglobin 85 per cent.
April 22, 1912	Urine: no haemoglobin, no bile Blood:red cells, 3,250,000; haemoglobin 49 per cent.	April 17, 1912	Urine contains haemo- globin and bile-pig- ment Blood: red cells, 4,470,- 000; haemoglobin 73 per cent.
April 23, 24, 25, 1912	Urine: no bile-pigment Blood:red cells, 3,040,000; haemoglobin 42 per cent.	April 18, 1912	Urine: no haemoglobin, but trace of bile
April 24, 1912		April 19, 1912	Urine: no bile, no albu- min Blood: red cells, 4,930,- 000
April 25, 1912	Blood:red cells, 2,910,000; haemoglobin 42 per cent.		

that, if haemoglobin be supplied to it in excess of a given amount, jaundice will result, but that, conversely, haemoglobin supplied to it in quantities less than this amount will not give rise to jaundice. Now it may well be that

in an animal rendered anaemic either by bleeding or by insufficient blood formation the daily blood-cell destruction may be less than in the normal animal and hence the liver be further from its normal saturation point for haemoglobin. In such an animal more haemoglobin could be liber-

TABLE XXII

EFFECT OF HEMOLYTIC SERUM ON SPLENECTOMIZED DOGS WITH NORMAL RED CELL COUNT

Date	Four day splenectomy	Date	Sixty-six day splenectomy
April 17, 1912	Weight, 7,850 gm. Splenectomy	Feb. 10, 1912	Splenectomy
April 21, 1912	Urine: no albumin, no bile Blood: red cells, 5,880,000; haemoglobin 102 percent. Fragility: 0.3+; 0.45- 11.25 A. M. Received in vein 0.25 c.c. serum per kilo.	April 16, 1912	Weight, 12,680 gm. Urine: faint trace of albumin, no bile Blood: red cells, 5,230,- 000; haemoglobin 83 per cent. Fragility: 0.25+; 0.35- 5.20 P. M. Urine: (by catheter) con- tains large amount of bile-pigment and faint trace of albumin Blood: red cells, 5,330,000; haemoglobin 93 percent.
		12 M.	Received in vein 0.25 c.c. serum per kilo.
		2.45 P. M.	Urine: trace of bile-pig- ment
		3.50 P. M.	Blood: red cells, 5,310,- 000; haemoglobin 84 per cent.
April 22, 1912	Urine: trace of bile Blood: red cells, 5,260,000; haemoglobin 81 percent.	11.00 P. M.	Urine: moderate amoun of bile-pigment
April 23, 1912	Urine: faint trace of bile	April 17, 1912	Urine: moderate amount of bile-pigment
April 24, 1912	Urine: no albumin, no bile Blood: red cells, 4,800,000; haemoglobin 70 percent.		Blood: red cells, 4,830,- 000; haemoglobin 79 per cent.
April 25, 1912	Blood: red cells, 4,510,000; haemoglobin 80 percent.	April 18, 1912	Urine: no bile-pigment. Blood: red cells, 4,500,- 000
		April 19, 1912	Urine: no bile.

ated into the circulation as the result of a single insult and be removed by the liver without exceeding the saturation point of the liver and thus jaundice would not occur.

It is therefore at once evident that if it is the presence of an anaemia, subsequent to the splenectomy, which is of

importance in preventing the development of jaundice, then haemolytic serum administered to an animal soon after splenectomy, before anaemia has developed, or long after splenectomy, when the blood has again returned to normal, should induce the appearance of the bile-pigments in the urine. Both these experiments were performed and the results tabulated in Table XXII.

In both of these splenectomized animals bile-pigments appeared in the urine, and a trifle more abundantly in that animal which at the beginning had the higher haemoglobin and red blood-cell count. Thus anaemia would appear also to be a factor in lessening the tendency to jaundice after administration of a haemolytic agent.

(4) INFLUENCE OF THE INCREASED RESISTANCE OF THE RED CELLS

In neither of the splenectomized animals in Table XXII was the jaundice quite so marked as in the control animal given the same dose of the same serum (see Table XXI), and in neither did the haemoglobinuria occur that was observed in the control. The rate of fall of haemoglobin and red blood-cells in these animals shows that the rate of blood destruction in the control animal was much greater than in either of the splenectomized animals. In the animal splenectomized four days the eventual blood destruction equalled that of the control, but it occurred much more slowly; and in that splenectomized for sixty-six days the amount of blood destruction was relatively slight. This constitutes a peculiarity in the reaction of a splenectomized dog to haemolytic agents which necessitates detailed discussion. The splenectomized animal may show an eventual

blood destruction, following haemolytic agents, less than, equal to, or greater than, the controls, but almost always the rate of blood destruction is slower. We believe that at least one factor although not the only one, in this phenomenon is the influence of the increased resistance of the red blood-cells. In confirmation of this it may be noted that in the three animals under discussion the rate of blood destruction was proportionate to the fragility of the red blood-cells. Such increased resistance of the red blood-cells has been shown to be characteristic of all splenectomized dogs. Consideration of the results obtained upon introduction of free haemoglobin into the circulation at various rates indicates at once the importance of a slower rate of blood destruction which must lead to a diminished tendency both to haemoglobinuria and to jaundice. Hence the increased resistance of the red blood-cells, in that it causes a slower rate of blood destruction after administration of haemolytic agents, is a third factor to be considered in any attempt to explain the lessened tendency in these animals to haemoglobinuria and jaundice.

Anæmia is, of course, no longer operative in animals splenectomized for long periods of time in which the blood picture has returned to normal. In such the increased resistance of the red cells, which persists indefinitely—our longest observation covers twenty months—must be a factor which coöperates with the mechanical factor previously discussed to limit the degree of jaundice. In such animals a faint trace of jaundice—never as much as in the control animal—is not unusual.

This persistence of the lessened tendency to jaundice one year after splenectomy is shown in the following table:

TABLE XXIII
EFFECT OF HÆMOLYTIC SERUM ONE YEAR AFTER SPLENECTOMY, WITH
CONTROL

Time	One year after splenectomy (dog 42)	Time	Control (dog 43)
Before injection	Weight, 6,400 gm. Urine: no albumin, no bile	Before injection	Weight, 6,615 gm. Urine: no albumin, no bile
1st injection	Received in vein 0.5 c.c. serum per kilo.	1st injection	Received same dose of same serum as dog 42
After injection 1-3 days	Urine: trace of albumin; faint trace of bile	After injection 1 day	Urine: trace of albumin; well marked bile test
3 days	Received in vein 1 c.c. of same serum per kilo.	2-3 days	Urine: no bile
2d injection	Marked haemoglobinuria	2d injection	Received same dose of same serum as dog 42
4 days	Faint haemoglobinuria;	4 days	Marked haemoglobinuria
5 days	doubtful bile test	5 days	Moderate haemoglobin- uria; marked bile test
6 days	Faint haemoglobinuria; faint but definite bile test	6 days	Faint haemoglobinuria; large amount of bile
	Died. No evidence of jaundice at autopsy	7 days	Urine: no haemoglobin; deeply bile-stained Chloroformed. At au- topsy general bile staining of tissues

We may conclude, therefore, that three factors are concerned in the decreased tendency to jaundice when hæmolytic agents are administered to splenectomized animals. The most important is the mechanical factor, the disturbance of the spleen—liver circulation; the second, always present, is the increased resistance of the red cells. These two factors apparently always work together. A third possible factor, not always clearly demonstrable, is that of anæmia.

(5) HÆMOLYTIC POWER OF SPLENIC EXTRACTS

The histologic evidence of the destruction of erythrocytes by phagocytic cells of the spleen has naturally suggested the possibility of the liberation by these cells of a

ferment capable of acting extracellularly. If it could be demonstrated that such a free haemolysin is present in the spleen we would have at once an adequate explanation of the decreased tendency to jaundice in the splenectomized animal, for with the spleen absent fewer red cells would be destroyed and less haemoglobin sent to the liver for the elaboration of bile-pigment. As this hypothesis has been made the basis for splenectomy in haemolytic anaemias, we have gone into this question in some detail. The literature of the subject shows that during the past few years several investigators have tested the influence of such spleen extracts upon red cells. The methods employed, based on the technique of Korschun and Morgenroth,²²⁵ are very similar, but the results obtained have been very contradictory. Korschun and Morgenroth found in several organs a haemolytic substance of unknown origin, coctostabile and soluble in alcohol, which did not arise from constituents of the blood-serum and was in no way peculiar to the spleen. Nolf,³¹⁷ on the other hand, found that the haemolytic power of splenic extract was distinctly greater than that of the liver, mesenteric lymph-nodes, or kidneys, but only slightly more than that of the lung. This haemolytic substance was specific for the species and was destroyed at 100° C. Achard, Foix, and Salin,³ repeating these experiments, showed that the final solution was strongly acid, presumably as the result of bacterial action, and that control tests made with precaution as to asepsis were uniformly negative. Widal, Abrami, and Brulé,⁴⁶⁹ in similar experiments, could get no haemolysis with fresh extracts used on the day they were prepared; sometimes, also, extracts 24 to 48 hours old were without effect. From these results they conclude that the haemolytic substance is not a true haemo-

lysin, but the product of cell autolysis. Iscovesco and Zacchiri¹⁹⁶ have shown that after placing the mixture of pulp and saline solution in the thermostat for fifteen to twenty hours the filtered extract, on the addition of red cells and after standing two and one-half hours in the thermostat, exhibits 2.5 per cent. to 8 per cent. haemolysis, as determined by the Dubosc colorimeter, and conclude that the haemolytic power of splenic extracts is unimportant. Weill⁴⁶⁴ found a weakly haemolytic substance in extract of spleen that was inactivated at 56° and reactivated with guinea-pig serum. This was more powerful than a lymph-node extract prepared in the same way, but much less powerful than the extract obtained from the spleen by long maceration. The latter was not destroyed below 80° C., and its action was hindered by adding fresh serum. Extracts from lymph-nodes prepared in the same way showed only slight haemolytic action, and those from other organs were negative. Banti²⁹ and Furno¹³⁴ state that fresh extracts of the normal spleen sometimes have no haemolytic action and sometimes a weak action which is increased on standing 24 to 48 hours on ice and is not destroyed by heating to 60° or even 100°. They consider it a cytohaemolysin, normally present in the spleen in small amounts and much increased after the administration of haemolytic agents. Thus we find that Nolf, Weill, Banti, and Furno find splenic extracts to have a haemolytic action greater than that of other organs. Achard, Foix and Salin, and Widal, Abrami and Brûlé, on the other hand, fail to find any haemolytic action of the fresh extract, and think it occurs only after autolysis or bacterial decomposition of the spleen.

Our experiments were made with extracts from the

spleens of three dogs. The technique described by Nolf was followed in the main, with several additions in the way of control experiments. On washing through the aorta it was found that the technique which will give a blood-free kidney or liver will not render the spleen bloodless. Various expedients were tried, therefore, to secure a haemoglobin-free extract. It was found that if the spleen, after washing through the aorta, was cut in small pieces and pounded with a pestle against a wire-meshed sieve placed in a mortar, with the aid of frequent washings with salt solution, a blood-free white mass was obtained consisting partly of reticulum and partly of adherent splenic pulp. (In Table XXIV this is called "Extract A.") As it was possible that the haemolytic substance might not be retained, or in only small amounts, in this fraction, extracts were also made from that part of the spleen that was mashed through the sieve. This residue was, of course, distinctly blood tinged, so that it was necessary, in order to remove the blood, to mix it with distilled water, centrifuge, discard the supernatant fluid, and repeat the process until colorless tissues were obtained. (In Table XXIV this is termed "Extract B.") In each case the material thus obtained was mixed with double the amount of salt solution and placed in the refrigerator. Tests were always made with extracts one or two hours old—a small portion being filtered off for this purpose—and in two instances also after eighteen and twenty-four hours. Control tests were made in one experiment with extracts of liver and mesenteric lymph-nodes. As it was possible to wash these organs free of blood before removal from the body, extracts were easily obtained by grinding the tissues in sand with mortar and pestle and placing them as before in the ice-

chest with double the amount of salt solution. In two experiments the tests were made on the corpuscles of the animal furnishing the spleen; in one the corpuscles of another dog was used without a difference in result. The preparation of the washed red blood-corpuscles, the dilutions, incubation, and so forth, were according to Nolf's technique. Each tube contained 0.1 c.c. of washed dog's corpuscles with varying amounts of splenic extract made up to 2 c.c. with normal salt solution. Controls were made with normal salt solution and distilled water. The results are presented in Table XXIV.

TABLE XXIV
THE HÆMOLYTIC POWER OF EXTRACTS OF SPLEEN AND OTHER ORGANS

Character of extract	Amount of splenic extract in c.c.								Salt solution control	Distilled water control
	1.95	1.5	1.0	0.5	0.3	0.2	0.1	0.05		
1. Dog 1. Fresh spleen extract A . . .	—	V.S.	0	0	0	0	0	0	0	C.
2. Dog 2. Fresh spleen extract A . . .	—	0	0	0	0	?	0	0	0	C.
3. Same. Extract B. . . .	—	0	0	0	0	0	0	0	0	C.
4. Same. After extraction in ice chest for 24 hours . . .	—	V.S.	V.S.	0	0	0	0	0	0	C.
5. Dog 3. Fresh spleen extract A . . .	V.S.	0	0	0	—	0	0	0	0	C.
6. Spleen extract A after extraction in ice chest for 24 hours . . .	—	0	0	0	0	0	0	0	0	C.
7. Fresh spleen extract (boiled)	0	0	0	0	—	0	0	0	0	C.
8. Fresh liver extract	0	0	0	V.S.	—	0	0	0	0	C.
9. Fresh mesenteric lymph node extract	0	0	0	0	—	V.S.	0	0	0	C.
10. Mesenteric lymph node extract after extraction in ice chest for 24 hours . . .	—	0	0	?	V.S.	M.	V.S.	0	0	C.

0 = no hæmolytic; ? = doubtful hæmolytic; V.S. = very slight hæmolytic; M = marked hæmolytic; C. = complete hæmolytic; — = no test.

From these observations it would appear that fresh extracts of spleen are devoid of definite hæmolytic action. Occasional irregular results, not to be explained, are found, but these occur likewise in the control extracts of liver

and mesenteric lymph-nodes. Extracts 24 hours old, prepared at low temperature, show little or no increase in haemolytic activity. Boiled splenic tissue, extracted in the cold for 24 hours, is inert.

From these results we are forced to the opinion that our present methods of demonstrating haemolysis *in vitro* are not adapted to proving the presence of a haemolysin in fresh extracts of the normal spleen, and we are inclined to agree with those who believe the reported positive results to be due to the products of organ autolysis or bacterial action. Moreover, we believe that no accuracy can be claimed for work done with extracts which are not free at the outset of both red cells and haemoglobin. We do not, however, deny that the spleen contains a haemolytic body. The histological evidence of the presence in the spleen of cells which engulf and destroy red cells, and which presumably cause this destruction through a ferment action, forbids such an opinion. These cells may contain a haemolysin which may act, as Banti suggests, intracellularly, or, when they are destroyed, extracellularly, but of this we have as yet no proof.

(6) INFLUENCE OF FATTY ACIDS AND LIPOIDS IN HÆMOLYSIS

Another theory of haemolysis is that which involves the action of fats and lipoids and with which are associated the names of Joannovics and Pick.²⁰¹ This is based on the fact that larger doses of toluylenediamine are necessary to cause icterus in splenectomized dogs than is the case in normal animals and also upon the fact that toluylenediamine is not haemolytic *in vitro*. It is evident that if toluylenediamine is hemolytic *in vivo* and not *in vitro* some factor other than the drug itself is concerned. This cannot be the

spleen alone, for in splenectomized dogs hæmolysis can be produced, if large enough doses—two to three times the usual amount—are used. Joannovics and Pick found that in toluylenediamine poisoning they could obtain from the liver a hæmolysin soluble in ethyl and methyl alcohol and in ether and acetone, and resistant to heat. Between acute and chronic poisoning were found certain differences. In chronic intoxication the hæmolysin was influenced by the absence of the spleen, that is, liver extracts from splenectomized animals were less active. In acute poisoning the hæmolytic power of the liver extract was not influenced by the absence of the spleen. In these livers were found palmitic, stearic, and oleic acids. Hæmolytic bodies similar to those described by Joannovics and Pick have been found in human livers in phosphorus poisoning and acute yellow atrophy (Jakoby).¹⁹⁹

Maidorn,²⁶⁵ whose experiments are in general confirmatory of the observations described, finds that the hæmolytic substance occurs only in the presence of fatty degeneration. Eppinger¹⁰⁴ and King,²¹³ on the basis of these various observations, studied the fat content of the blood of normal and splenectomized animals and of a number of individuals suffering from diseases characterized by hæmolysis, in order to determine whether any of these conditions were associated with an increase or decrease in the blood, of unsaturated fatty acids. According to their results, the blood of the dog after splenectomy shows an increase of the total fats and, as a rule, of cholesterin, with a lowering of the iodine figure representing the unsaturated fatty acids; the figures for the cholesterin esters were variable. Typical experiments from King's papers are presented in Table XXV (a).

These figures naturally raise the question of the pos-

sible influence of cholesterolin and the unsaturated fatty acids in changing conditions of haemolysis after splenectomy. The change in fatty acids is especially important,

TABLE XXV (a)

BLOOD FAT OF THE DOG BEFORE AND AFTER SPLENECTOMY (FROM KING²¹)

Whole blood	Total fat*	Cholesterin*	Cholesterin esters*	Iodine number
Dog 4				
With spleen.....	6.601	0.572	0.306	110.9
Without spleen, 14 days....	9.085	0.617	0.390	12.3
Without spleen, 2 months.....	8.328	0.625	0.309	60.1
Dog 9				
Serum—with spleen.....	4.449	0.120	0.132	101.2
Serum—without spleen....	9.815	0.318	0.348	26.0
Corpuscles—with spleen....	3.883	0.393	0.048	53.8
Corpuscles—without spleen	6.377	0.192	0.262	53.3

*Figures represent grammes per 1000 c.c. of blood.

TABLE XXV (b)

BLOOD FAT OF THE DOG BEFORE AND AFTER SPLENECTOMY (DUBIN AND PEARCE⁹⁹)

Dog	Before splenectomy		Ten days after splenectomy		Remarks
	Total* fats, gm.	Iodine* number	Total fats, gm.	Iodine number	
15-55 Serum.....	2.6	51.6	2.82†	51.5	Blood drawn into oxalate
Cells.....	2.98	43.7	2.42	52	
15-67 Serum.....	2.85†	51.6	2.83†	51.5	Blood drawn into oxalate
Cells.....	2.43	53.4	2.43	49.4	
15-76.....	5.63	47.6	5.99	47	Blood drawn into alcohol
15-75.....	5.25	49	5.63	48.2	Blood drawn into alcohol
16-23.....	5.73	47.4	6.036	49.6	Blood defibrinated
16-4.....	7.6	70.1	7.71	65	Blood defibrinated

* The iodine number is calculated on the basis of the total amount of fatty extract found in 100 c.c. of blood; the total fats are calculated per 1,000 c.c. of blood.

† As some haemolysis took place while separating the cells from the serum, there may be some inaccuracy in the relative values given for cells and serum.

as Eppinger found that in a variety of clinical conditions characterized by excessive haemolysis there occurs an increase of urobilin in the stools which goes hand in hand with an increase in iodine number of the blood, and that after

splenectomy, urobilin sinks to normal as the iodine number lessens. Such observations have obviously a definite relation to the increased resistance of the red cells and the decreased tendency to jaundice we have found constantly in the dog after splenectomy. As a preliminary, therefore, to experimentation along this line, we repeated the studies of Eppinger and King in so far as they related to the total fat and unsaturated fatty acids of the blood before and after splenectomy.⁹⁹ Our results indicate that splenectomy has no influence on the blood fat and are therefore not in accord with those obtained by Eppinger and King (see Table XXV [b]).

Just how the spleen might influence changes in the fat and lipoid content of the blood it is difficult to see, but, in view of the experiments of Anitschkow⁷ demonstrating the deposition of anisotropic fats in large quantities in the spleen of the rabbit after feeding cholesterol and egg-yolk, it is conceivable that the spleen stores or elaborates a lipoid concerned directly or indirectly in haemolysis, and that changes in this function may be a factor in the diminished jaundice caused, after splenectomy, by a haemolytic agent. This hypothesis is sufficiently attractive to justify, in view of the contradictions between our work and that of Eppinger and King, a delayed opinion, in the hope that further experimentation may throw more light on this complex problem. In this connection it is of interest that Kolmer and Pearce²²³ have shown that splenectomy alone has apparently no influence upon the property in normal rabbit and dog serum of fixing or absorbing complement with various non-specific lipoidal antigens. Such effects as were observed were attributable to the effect of the anaesthetic and not to the splenectomy.

CHAPTER IV

CONCERNING THE SUPPOSED REGULATORY INFLUENCE OF THE SPLEEN IN BLOOD DESTRUCTION AND REGENERATION

B. IN RELATION TO ANÆMIA: (1) A COMPARISON OF THE ARTERIAL AND VENOUS BLOOD OF THE SPLEEN. (2) THE INFLUENCE OF SPLENIC EXTRACT UPON BLOOD FORMATION. (3) THE INFLUENCE OF FEEDING SPLEEN TO SPLENECTOMIZED ANIMALS. (4) THE REPAIR OF AN ARTIFICIALLY PRODUCED ANÆMIA IN SPLENECTOMIZED ANIMALS. (5) THE INFLUENCE OF THE SPLEEN ON IRON METABOLISM.

Many efforts have been made to show that passage through the spleen alters the red cells and renders them more susceptible to haemolysis. The results of work along these lines are very contradictory, but, as our problems demanded a first-hand knowledge of the subject, we have made a number of experiments²²⁸ in this field.

(1) A COMPARISON OF THE ARTERIAL AND VENOUS BLOOD OF THE SPLEEN

Much of the early work on this subject is not only contradictory, but was done before the development of the exact methods of blood examination with which we are now familiar. Thus Virchow⁴⁵⁵ found fewer red cells in the blood of the splenic vein than in that of the artery; while Malassez and Picard,²⁶⁸ and Emilianow¹⁰³ report

the opposite. On the other hand, later investigators, Vulpius⁴⁶⁰ and Paton, Gulland and Fowler,³³⁰ have found no constant or noteworthy differences.

Considering the spleen as a possible leucoblastic organ, numerous early observers found relatively more leucocytes, especially so-called young forms, in the blood emerging from the spleen than in that entering it. Tarchanoff and Swaen,⁴²⁷ as also Virchow,⁴⁵⁵ could not find any noteworthy difference, whereas Paton, Gulland, and Fowler³³⁰ noted a constant diminution in the number of leucocytes in the splenic vein as compared with the general circulation. In this connection Bulgak,⁶² who describes an increase in leucocytes in the splenic vein, states that this is true of the venous blood of all parenchymatous organs. Freyer¹³⁰ concludes, from his comparative counts, that the spleen has nothing to do with blood formation.

All this work, of course, refers to mature animals. It is generally accepted that in fetal life the spleen has the power of extensive blood formation, and several reports are at hand to show that in the adult the spleen may undergo, in the presence of injury to the bone-marrow, a myeloid metaplasia;⁹⁶ that is, that it can regain its fetal function under pathological conditions. Whether or not the spleen may exert this power of blood formation in the adult under normal conditions is very doubtful, though to some observers it is still an open question.

On the other hand, although the spleen certainly destroys red blood-cells (as is evident from the presence in it of large cells, phagocytic for erythrocytes, which are present in increased numbers under certain pathological circumstances), there is still some doubt as to whether this destruction by phagocytosis is the only method of red-cell

disintegration. Many investigators claim that the erythrocytes, in their passage through the spleen, are so acted upon by some unknown substance as to become more susceptible to haemolysis.

This is the basis of Bottazzi's⁵³ haemocatatonistic theory, which has recently received support from Banti²⁹ and his colleague, Furno.¹³⁴ In the course of an investigation of haemolytic splenomegaly, they studied normal animals and those receiving haemolytic serum and came to the conclusion that free haemoglobin can be demonstrated in the blood of the splenic vein both in normal animals and in those receiving haemolytic serum. Sometimes they found it in the blood of other vessels, but always in less amounts than in the splenic vein. These findings they consider as evidence of haemolysis in the spleen. Also the red blood-cells of the splenic vein were found to be less resistant to hypotonic salt solution than were those of the general circulation. On the other hand, investigations by Chalier and Charlet⁷¹ on the resistance of red cells in the splenic artery and vein gave very different results. Although they found that venous blood in general was slightly less resistant than arterial blood, in the splenic system this was reversed, so that the blood of the splenic vein was more resistant than that of the splenic artery and much more so than the blood of other veins. Hammarsten also, according to Gabbi,¹³⁵ found that the splenic vein blood was more resistant than the arterial.

In the observations of Banti and Furno, the reference is to free haemoglobin in the serum and not to the increased haemoglobin content of venous or splenic blood described by several investigators. This claim is most surprising, in that they state that the dissociated haemoglobin of the

serum ("emoglobin disciolta dal siero") is not only always present in the splenic vein of normal animals, but sometimes in sufficient quantities to be measured by a Sahli haemoglobinometer. It is to these observations that we have given especial attention in our work.

METHODS.—From dogs under ether anaesthesia blood was obtained directly as it flowed from the splenic artery and the splenic vein. Great care was exercised to disturb the vessels and the organs as little as possible, as it has been shown by Grigorescu¹⁵⁵ and Pribram³⁶¹ that the cell content of the blood may be greatly increased by congestion of the spleen. From a nick in the wall of one of the branches of the artery or vein fresh blood was drawn directly into Thoma blood-counting pipettes and the capillary tube of a v. Fleischl haemoglobinometer. From another branch blood was withdrawn by a syringe and immediately distributed to tubes containing different strengths of hypotonic salt solution designed to test the resistance of the red cells. Some of the blood was also set aside for similar tests with washed cells. For the determination of the presence of free haemoglobin in the serum blood was collected in three ways: (1) in a paraffined centrifuge tube, (2) in a tube containing potassium oxalate, and (3) by drawing it directly into tubes through capillary points, which were then sealed. All three samples were centrifuged and the serum examined for haemoglobin by visual inspection and the spectroscope. Smears for differential counts were made at times from the blood flowing directly from the vessel, and at times from a drop from the syringe. Finally, tests for reticulated or skeined (young) red blood-cells were made. This was done by letting a few drops of blood fall into a solution of brilliant cresyl blue, and, after

standing fifteen or twenty minutes, the skeined forms in proportion to the unskeined or mature forms were counted in fresh smears. For the purpose of controls, blood from the femoral vein and from the capillary circulation (by puncture of the skin) was occasionally collected.

RESULTS.—The figures²²⁸ for the red and white cells, differential counts, and total haemoglobin in a series of five dogs show that, so far as these estimations are concerned, the blood of the splenic vein does not differ greatly from that of the artery. The variations are not uniformly on one side, and are all within the limit of error inherent in the methods of blood examination.*

It is of interest that in six of eight animals the red cells of the vein showed more or less marked anisocytosis and

* Since these observations were made, Morris²⁰¹ has published similar studies on the cat. He comes to the conclusion that the spleen plays a definite rôle in the formation of the red blood-cells. This conclusion is based on the counting of the red cells in the splenic artery and vein, the number in the latter being one to four million greater than in the artery; in one animal, for example, 4,400,000 per c.mm. in the artery as against 9,120,000 in the vein. Morris's technic appears to differ from ours only in that he collected his blood from the stagnant stream between two clamps, while we took the free-flowing blood as it passed out of a small nick in the vessel wall. Comparative tests which we have made, since his publication, of stagnant and flowing splenic vein blood show that sometimes in the former the count may be one to three million higher than in the latter; in other instances, it is the same. We are therefore inclined to think that Morris's high counts may be due to mechanical causes, especially as in repetitions of our earlier work, three dogs being used, we found a variation between the artery and vein of never more than 500,000 cells. The higher count occurred twice in the venous blood and once in the arterial blood—variations well within the limit of error of blood-counting methods.

inequality of staining, which were not seen to the same degree in the blood of the artery. Polychromatophilia was about equal in blood of the artery and the vein. In two of the eight experiments a few normoblasts were found in the splenic vein blood only. Control smears from the femoral vein of four dogs showed changes in the red cells about equal to that of the splenic vein, indicating that these changes are characteristic of venous blood in general rather than any specific change caused by passage through the spleen.

In regard to the presence of free hæmoglobin in the serum, if we had depended on one tube only we would have occasionally found apparent hæmoglobinæmia, both in the general circulation and in the splenic vein; but as in every set of three tubes, in a series of seven dogs, at least one was free of hæmoglobin, we cannot support the view that free hæmoglobin in demonstrable amounts is present normally either in the splenic vein or the general circulation of the dog. Our experience forces us to the conclusion that the findings of other investigators are due to haemolysis after collection or are dependent upon the method of separating the serum.*

As regards the resistance of the red cells of the vein

* During the past few years, in connection with investigations on coagulation of blood, Abderhalden's theory of protective enzymes, Folin's microchemical methods, and the phenomena of anaphylaxis, much time and attention has been given in this laboratory to the collection of plasma and serum from the dog and rabbit. In our experience careful collection always yields these fluids free of hæmoglobin; discolored sera we have always regarded as due to errors in the method of collection. With our experience in mind, we cannot support the statements of Banti and Furno that free hæmoglobin, in amounts sufficient to be recognized, occurs normally in the serum.

as compared with the artery, tests were made on eight dogs: in five no difference was found; in the other three the venous corpuscles were slightly less resistant. Two control tests with cells from the femoral vein showed these to have the same resistance as cells of the splenic vein blood. The question arises, therefore, as to whether the differences described heretofore are not those of arterial and venous blood in general.

In seven comparative tests for skeined or reticulated red corpuscles these were found to be more abundant in five in the splenic vein and in two in the artery; the differences were never very striking. Five controls from the femoral vein corresponded more closely to the splenic artery counts than to those from the splenic vein.

As a result of these various observations we conclude that the slight differences between the arterial and venous blood of the spleen are within the limits of error inherent in the methods of blood examination, and are not to be explained by a peculiar action of the spleen. In some instances peculiarities shown by the splenic venous blood are common to the venous blood of the general circulation. Banti and Furno's observation concerning the presence of free haemoglobin in the blood of the splenic vein is not confirmed.

2. INFLUENCE OF SPLENIC EXTRACT UPON BLOOD FORMATION

Nearly all investigators grant the spleen a function in the destruction of red cells; and some ascribe to it a part in red-cell formation. This latter view is based largely on the fact that in fetal life red cells are formed in the spleen, and that under pathological conditions myeloid

metaplasia may occur. We have, however, no satisfactory evidence that this function is continued under normal conditions beyond a short period after birth. Some work has been done with splenic extracts, as by Danilewsky,⁸⁷ to show that the spleen contains a substance which stimulates the formation of red cells in the bone-marrow. Danilewsky found a surprising increase in hæmoglobin and red blood-cells after a single subcutaneous or intraperitoneal injection of extracts of spleen. This increase reached its height in from three to seven days and continued as long as the experiment lasted, usually eight days. In dogs with a dietary anæmia,* splenic extract caused an even greater rise; for example, of 40 per cent. hæmoglobin and almost 2,000,000 red cells. This influence of the splenic extract was not destroyed by heating. Danilewsky assumed that his results were due to a stimulation of the bone-marrow. As Danilewsky's work is uncontrolled by injection of other organ extracts, we have repeated his experiments. Silvestri⁴⁰⁸ records a single observation in which a dog, presumably dying from anæmia, was apparently saved by the injection of splenic extract. In this connection it must also be noted that the clinical literature of this subject contains several reports of the use of extracts of spleen and bone-marrow with good results in the treatment of anæmia.

METHOD.—We have tested the effect of splenic extract on four dogs, using as controls extracts of other organs similarly prepared and extracts of erythrocytes.

The usual examinations of the blood were made, as were also determinations of the resistance of the erythrocytes to hypotonic salt solution and of the percentage of

* Anæmia due to a diet of rice only; red cells fell to 3,980,000.

skeined cells. As a rule, two counts were made before injection and daily counts after the injection until the blood picture had returned to normal, usually a period of from three to four days. Extracts were prepared from organs removed aseptically from dogs bled to death under ether anaesthesia. The finely chopped organ was ground in a sterile mortar to a homogeneous pulp and extracted with double the volume of salt solution for two hours in the ice-chest. Ten cubic centimetres of the filtered extract was injected intraperitoneally into dogs of about the same weight. As the splenic extract contained a considerable amount of blood, it was necessary to use as control defibrinated blood (10 c.c.), diluted with normal salt solution (1 to 20), in order to determine the possibility of the rise in red cell count being due to the influence of some constituent of the red cells. In no case did peritonitis or other infection result from the injection.

The result²²⁸ in one of these experiments is shown in Table XXVI.

TABLE XXVI

EFFECT ON THE BLOOD PICTURE OF INJECTIONS OF SPLENIC EXTRACT

Date (1914)	Hæmoglobin	Red blood cells
Feb. 6	102	5,250,000
Feb. 7	101	5,650,000
Feb. 8	(10 c.c. splenic extract No. 16 injected) 110	6,500,000
	(15 c.c. of same extract injected) 110	7,040,000
Feb. 9	105	6,800,000
Feb. 11	96	5,330,000
Feb. 12	95	5,290,000
Feb. 13	(15 c.c. splenic extract No. 88 injected) 101	5,700,000
	(10 c.c. of same extract injected) 104	6,880,000
Feb. 14	98	5,860,000
Feb. 16	96	5,120,000
Feb. 19	106	5,540,000

This experiment shows that intraperitoneal injection of splenic extract causes a sharp rise in haemoglobin and red cell count, lasting only one or two days. This rise is repeated on reinjection of either the same or another splenic extract.

In each of three other experiments with splenic extract an increase in the number of red cells was obtained, but this increase was not always as marked as in the experiment presented; it was nevertheless always greater than that caused by the use of control extracts of liver, kidneys, or blood.

The study of the resistance of the red cells in these experiments may be dismissed with the statement that no noteworthy differences were found after injection of any extract. The skeined cells also showed no constant change. We had hoped that, as the latter are supposed to be young forms of erythrocytes, they would be found to be increased after the injection of splenic extract had caused a rise in the red cell count. Only once, however, in which instance the percentage rose from 0.5 to 2, was this noticed. On the other hand, in two experiments they were not found at all in the blood after injection.

Intraperitoneal injection of splenic extract is usually followed by an increase in the total number of leucocytes, consisting chiefly of the polymorphonuclear forms. A similar rise occurred in one of three experiments with liver and kidney, and in one of two with defibrinated blood. Several grades of "transitional cells" appeared in increased numbers. Eosinophiles were present in increased numbers in two of the four dogs receiving splenic extracts, but were also definitely increased in two of the five controls receiving other organ extracts.

It would appear, therefore, that the intraperitoneal injection of saline extracts of fresh spleen constantly causes a sharp increase in red-cell count and haemoglobin content. The rise is evanescent, lasting but one or two days, and may be followed by an equally evanescent drop below normal. Similarly prepared extracts from other organs fail to give this rise. No noteworthy change is found in the resistance of the red blood-cells to hypotonic salt solutions or in the number of skeined or reticulated erythrocytes after the injections of the various organ extracts.

A temporary increase of polymorphonuclear and transitional leucocytes usually follows the use of spleen extract, but may occur also, but less frequently, after the injection of liver and kidney.

The constant increase of red cells in the peripheral circulation after injection of spleen, in view of the tendency to anaemia following splenectomy, suggests that the spleen normally may exert a stimulating effect upon the formation of red cells in the bone-marrow.

3. INFLUENCE OF FEEDING SPLEEN TO SPLENECTOMIZED DOGS

This study complements that just described in that spleen in large amounts was fed to splenectomized animals. The object was to determine whether through the influence of some necessary substance in the spleen the anaemia following splenectomy might be prevented. The procedure is, of course, analogous to thyroid feeding in insufficiency of the thyroid gland, and has an advantage over the injection of extracts in that it may be continued over long periods of time without the possibility of the complications occasionally occurring after injection. These

experiments, it was hoped, would show whether or not the spleen exerts some effect upon the hæmopoietic system through peculiar bodies analogous, perhaps, to those of an internal secretion. Thus if the anæmia following splenectomy depends upon the absence of a stimulus, furnished normally by the spleen, to the hæmopoietic system in general, or to some part of it, as the bone-marrow, the feeding of normal fresh spleen unmodified by heat or chemicals might supply this stimulus and there would then be no anæmia after the removal of the spleen.

METHOD.—Five dogs were used. Four of these were given a diet consisting of raw hashed beef spleen, lard, and cracker-meal in amounts estimated, according to the weight of each animal, to suit its caloric needs. Of these, three were splenectomized and one served as a control. As an added control, a splenectomized dog received a diet in which casein was substituted for beef spleen. The red cells and the hæmoglobin were estimated several times before splenectomy and afterwards counted twice a week for three weeks and then once a week for five weeks. No preliminary counts were made until a dog had been on the special diet for at least a week, and splenectomy was not performed until two weeks later.

RESULTS.—Of the three splenectomized dogs receiving spleen²²⁸ in the diet, one showed only a very slight decrease in red cells and hæmoglobin, but the other two developed the usual anæmia of splenectomy. Thus one with an initial red-cell count of 6,200,000 showed on the twelfth day 4,710,000 red cells, with return on the fifty-fourth day to 6,040,000. This animal received daily 150 grammes of beef spleen. The other dog receiving daily 275 grammes of spleen showed a change in red-cell content of about the

same degree. In the splenectomized dog not fed spleen the red cells fell from 5,500,000 to 4,210,000 on the 19th day, with return to 5,060,000 on the fifty-fourth day. In this dog the haemoglobin reached its lowest level (65 per cent.) on the twelfth day, and remained at about that point until the twenty-sixth day. In none of the other splenectomized dogs receiving spleen did the haemoglobin fall below 75 per cent. The normal dog, receiving 150 grammes of spleen daily, showed no change in the blood.

It is evident that in two dogs, despite the feeding of spleen, an anaemia was produced that ran a course very similar to that which we have previously shown to be the rule in splenectomized dogs. In view of these very definite results, the mildness of the anaemia in the third splenectomized dog cannot be considered as a sparing influence due to the feeding of spleen tissue.*

* The observation has recently been made by Lewis and Margot²⁴⁷ that the feeding of fresh sheep spleen to splenectomized animals gives rise to an acute intoxication that is sometimes fatal. This was frequently observed in splenectomized mice, and was noted in one of two splenectomized dogs. As an intoxication such as Lewis and Margot describe might be a disturbing factor in the study of anaemia, we have repeated our feeding experiments with beef spleen and have watched with special vigilance for the appearance of symptoms of intoxication. One dog was given a diet of raw beef spleen, lard, and cracker-dust for two weeks after splenectomy, and, as no untoward symptoms had developed in that time, pure beef spleen alone was given daily for three days. This he ate with relish, devouring over 1200 grammes of spleen during these three days, but at no time showed any signs of anorexia, nausea, vomiting, chills, or in fact any untoward symptoms whatsoever. Another dog was fed beef spleen, lard, and cracker-dust for ten days before and for two weeks after splenectomy. Neither this animal nor the normal control on the same diet showed any of the symptoms described as occurring after the feeding of sheep's spleen. The mod-

As, therefore, the feeding of fresh raw spleen to splenectomized dogs has no clearly defined influence in preventing the anæmia which usually occurs after splenectomy, a regulatory influence of the spleen, in this regard at least, cannot be assumed.

4. THE REPAIR OF AN ARTIFICIALLY PRODUCED ANÆMIA IN SPLENECTOMIZED ANIMALS

In the hope of throwing some light upon the problem of the regeneration of the blood in the absence of the spleen we have allowed animals to recover from the anæmia following splenectomy and have then caused anæmia in various ways and followed for long periods of time the changes in the blood picture.²²⁷ These studies have brought out the interesting fact that, despite the increased resistance of the red cells and the lessened tendency to jaundice, the anæmia caused in splenectomized animals by haemolytic agents may be of greater severity, as shown by direct blood examination, and always runs a longer course and has a longer period of repair than in the case of the normal dog. We have, therefore, the apparent paradox that an animal with more resistant corpuscles suffers a severer and more prolonged, although more slowly developing, anæmia than with corpuscles of the normal resistance. Before further discussing this apparent paradox, our methods and the results of detailed experiments may be presented.

METHODS.—The animals were given a simple but

erate anæmia which developed in the two splenectomized animals followed the course usually observed in splenectomized dogs. Considering, also, the three dogs previously studied by us, we therefore conclude that the phenomena observed by Lewis and Margot after feeding sheep's spleen do not occur in splenectomized dogs fed with beef spleen.

abundant diet, practically uniform in character and containing roughly the same constant amount of iron-yielding material. The iron in the diet was not, however, in this series of experiments, estimated quantitatively. After preliminary blood examinations had been made the spleen was removed aseptically, by a practically bloodless operation, under ether anaesthesia. The blood examinations were then continued, at first at short intervals and later at longer intervals. At various stages of the process of blood regeneration haemolytic immune serum or sodium oleate was administered, with, at the same time, the administration of an equivalent amount of the haemolytic agent to a normal animal serving as control. For the purpose of further control, animals were rendered anaemic by bleeding, in order that the regeneration of the blood in this type of anaemia might be contrasted with that due to haemolytic agents. The examination of the blood was continued after the production of anaemia, the intervals between examinations being gradually lengthened, and included the estimation of red cells, haemoglobin content, and the white cells, with differential counts of the latter and the determination of the resistance of the red cells to various percentages of hypotonic salt solution.

The two experiments given in detail in Tables XXVII and XXVIII represent the longest periods that animals have been observed after the administration of haemolytic serum. In all other experiments of this group, although the animals were carried for shorter periods, the general course of the anaemia was the same. The two tables are deemed sufficient, therefore, to illustrate the onset and repair in this type of anaemia. The first experiment (Table XXVII) is of interest chiefly in connection with

the question of the length of time necessary, in the splenectomized as compared with the normal dog, for a complete regeneration of the blood to occur after the destruction caused by hæmolytic serum. It will be seen that in

TABLE XXVII

EFFECT OF HÆMOLYTIC SERUM ON BLOOD PICTURE OF SPLENECTOMIZED DOG AND CONTROL *

Hæmolytic Immune Serum 61			Dog 53. Weight 7,780 gm. 0.25 c.c. hæmolytic serum per kilo. injected		
	Hæmoglobin, per cent.	Red blood cells		Hæmoglobin, per cent.	Red blood cells
Before	90	5,230,000	Before	92	6,200,000
2½ hours	72	3,800,000	3 hours	78	4,970,000
1 day	60	3,510,000	1 day	38	2,060,000
3 days	66	3,920,000	2 days	30	2,250,000
6 days	54	2,730,000	4 days	38	3,100,000
8 days	38	2,310,000	6 days	49	3,420,000
10 days	55	3,100,000	8 days	42	3,400,000
14 days	48	2,870,000	10 days	42	3,110,000
17 days	53	3,220,000	12 days	50	3,980,000
22 days	51	3,060,000	17 days	64	3,810,000
28 days	67	3,510,000	24 days	80	4,230,000
34 days	64	3,390,000	31 days	86	4,530,000
44 days	76	4,880,000	66 days	81	5,120,000
56 days	85	4,210,000	86 days	92	5,380,000
70 days	63	3,540,000	107 days	105	6,510,000
79 days	79	4,120,000			
100 days	86	4,010,000			
136 days	82	4,200,000			
200 days	110	6,200,000			

* The period intervening after splenectomy is indicated in parentheses after the number of the animal. The word "before" in the time column refers to the blood count made a short time before the injection of the hæmolytic agent and not to the blood count before splenectomy. Likewise hours and days in the same column are indicative of the length of time after such injection.

the normal dog the lowest figures were those of the second day, and that an approach to normal figures was evident after two months, but that the latter was not actually reached in the case of hæmoglobin until the third month and of the red cells until 107 days; on the other hand, the splenectomized animal exhibiting a more gradual fall did

TABLE XXVIII

RECOVERY FROM INJECTION OF HÆMOLYTIC SERUM OF SPLENECTOMIZED DOG AND CONTROL

Time	Ten months splenectomy, dog 59	Time	Control, dog 54
July 24, 1912	Splenectomy		
May 21, 1913	Weight 10,900 gm. Blood: red cells 5,200,000; hæmoglobin 86 per cent. Fragility: 0.3+, 0.45- Urine: no bile-pigment; trace of albumin; no casts 0.2 c.c. hæmolytic serum per kilo.	May 21, 1913	Weight 12,800 gm. Blood: red cells 5,400,000 hæmoglobin 104 percent. Fragility: 0.3+, 0.5- Urine: not obtained.
1st injection		1st injection	0.2 c.c. per kilo. of same serum Urine shows faint trace of bile after 10 min.
24 hrs. later	Blood: red cells 4,820,000; hæmoglobin 90 per cent.; serum free of hæmoglobin Fragility: 0.35+, 0.6- Urine: trace of albumin; trace of bile-pigment	24 hrs. later	Blood: red cells 5,050,000; hæmoglobin 94 per cent.; serum free of hæmoglobin Fragility: 0.35+, 0.6- Urine: trace of albumin; moderate amount of bile- pigment present
2d injection	Same dose of same serum repeated	2d injection	Same dose of same serum repeated
24 hrs. later	Blood: red cells 5,620,000; hæmoglobin 95 per cent. Urine: no hæmoglobin; faint but definite trace of bile-pigment; trace of albumin	24 hrs. later	Blood: red cells 4,880,000; hæmoglobin 98 per cent. Urine: slight hæmoglobin- uria; large amounts of bile-pigment
2d day.....	Blood: red cells 4,030,000; hæmoglobin 75 per cent. Urine: bile-pigment abun- dant but less than in dog 54	2d day.....	Blood: red cells 4,480,000; hæmoglobin 97 per cent. Urine: no hæmoglobin but large amount of bile-pigment
3d day.....	Blood: red cells 3,950,000; hæmoglobin 56 per cent. Urine: good bile reaction, but less than in dog 54	3d day.....	Blood: red cells 4,820,000; hæmoglobin 95 per cent. Urine: bile very abundant
4th day.....	Blood: red cells 3,580,000; hæmoglobin 52 per cent. Urine: trace of bile	4th day....	Blood: red cells 4,460,000; hæmoglobin 66 per cent. Urine: bile decreasing, but well marked
5th day.....	Blood: red cells 3,550,000; hæmoglobin 57 per cent. Urine: bile reaction slight	5th day....	Blood: red cells 4,720,000; hæmoglobin 74 per cent. Urine: abundant bile
7th day.....	Blood: red cells 3,660,000; hæmoglobin 52 per cent. Urine: no albumin or bile	7th day....	Blood: red cells 5,500,000; hæmoglobin 86 per cent. Urine: moderate bile; no albumin

THE SPLEEN AND ANÆMIA

TABLE XXVIII—*Continued*

Time	Ten months splenectomy, dog 59	Time	Control, dog 54
11th day....	Weight: 10,040 gm. Blood: red cells 4,250,000; haemoglobin 54 per cent.; leucocytes 10,400 Urine: no albumin or bile	11th day....	Weight 11,980 gm. Blood: red cells 5,800,000; haemoglobin 84 per cent.; leucocytes 17,000 Urine: no bile
14th day....	Blood: red cells 4,570,000; haemoglobin 72 per cent.; leucocytes 14,200 Urine: faint trace of bile	14th day....	Blood: red cells 5,640,000; haemoglobin 102 per cent.; leucocytes 18,200 Urine: faint trace of bile
17th day....	Blood: red cells 5,050,000; haemoglobin 86 per cent.; leucocytes 16,000 Urine: no albumin or bile	17th day....	Blood: red cells 6,100,000; haemoglobin 106 per cent. Urine: no albumin; very faint trace of bile
20th day....	Weight 10,415 gm. Blood: red cells 4,780,000; haemoglobin 79 per cent.; leucocytes 14,400	20th day....	Weight 12,365 gm. Blood: red cells 6,080,000; haemoglobin 109 per cent.; leucocytes 20,700
24th day....	Blood: red cells 5,440,000; haemoglobin 92 per cent. Urine: no albumin or bile	24th day....	Blood: red cells 6,120,000; haemoglobin 110 per cent.; leucocytes 13,200
27th day	Blood: red cells 5,020,000; haemoglobin 90 per cent.		
31st day....	Blood: red cells 3,480,000; haemoglobin 70 per cent.; leucocytes 13,400 Fragility: 0.275+, 0.55-	33d day....	Blood: red cells 6,200,000; haemoglobin 110 per cent.; leucocytes 13,200 Fragility: 0.325+, 0.55-
36th day....	Blood: red cells 4,870,000; haemoglobin 98 per cent. Urine: no albumin or bile		
40th day....	Blood: red cells 5,970,000; haemoglobin 102 per cent.		
46th day....	Weight 10,820 gm. Blood: red cells 5,140,000; haemoglobin 98 per cent. Fragility: 0.35+, 0.45-	46th day....	Weight 12,450 gm. Blood: red cells 6,060,000; haemoglobin 106 percent. Fragility: 0.35+, 0.55-

not reach its lowest figures until the eighth day, and still had a low haemoglobin content and red-cell count at the end of 136 days. On account of the long intervals elapsing in each case before the final count, the exact dates of return to normal cannot be given, but it is clear that one month after the control animal had reached its normal red-cell count the blood of the splenectomized animal was far from its normal state. At the end of 200 days, however,

the blood picture of this animal gave higher figures than before injection.

As these animals were of practically the same weight, and as the splenectomized dog received less serum than the normal dog, it is impossible to escape the conclusion that the absence of the spleen is an important factor in the chronicity of the anaemia and the slow repair.

In the second experiment (Table XXVIII) the same relative difference in the severity of the anaemia and the rate of recovery is seen, but in neither animal was the period of recovery as long as in the animals of the first experiment.

Some objection might be raised against the results in the first experiment, as this animal, despite the high initial count, had been splenectomized only twenty-six days, and the resulting slow repair might be due to a combination of a slow anaemia secondary to absence of the spleen and of the anaemia due to the haemolytic serum. This possible objection is removed by the results in the second experiment, in which the blood of an animal splenectomized nearly a year before had returned to normal before injection. The theory that the absence of the spleen is responsible for the slow blood regeneration is thus strengthened.

Sodium Oleate Anaemia in the Splenectomized Animal

On account of the advisability of determining the effect of another type of haemolytic poison, experiments were made with sodium oleate (see Table XXIX). This substance, unlike haemolytic serum, produces, as a rule, a transient anaemia, quickly repaired in the normal dog.

Here, also, it is seen that the anaemia was more severe and the repair slower in the splenectomized than in the

normal animal. In the first experiment the splenectomized dog's haemoglobin fell eventually on the thirtieth day to 54 per cent. and the red cells to 3,700,000, and it took forty-three days for the return to normal, whereas in the control receiving an equivalent amount of oleate the haemoglobin fell on the third day to only 93 per cent. and the

TABLE XXIX*
REPAIR OF SODIUM OLEATE ANÆMIA AFTER SPLENECTOMY, WITH CONTROL

Dog 24. Weight 9,650 gm. (5 months after splenectomy)				Dog 55. Weight 7,860 gm. Normal			
100 c.c. of 1 per cent. sodium oleate in vein				100 c.c. of 1 per cent. sodium oleate in vein			
	Hæmo-globin per cent.	Red blood cells	Leucocytes		Hæmo-globin per cent.	Red blood cells	Leucocytes
Before	89	5,650,000	10,600	Before	108	6,460,000	8,600
1 day	68	3,770,000	15,600	3 days	93	5,420,000	7,100
3 days	70	2,320,000	10,400	7 days	112	6,120,000	6,400
7 days	74	3,100,000	10,800	16 days	116	7,240,000	10,800
11 days	70	4,200,000	8,600	24 days	114	7,410,000	8,200
16 days	66	3,420,000	9,800				
19 days	66	3,760,000	10,200				
24 days	63	3,600,000	10,000				
30 days	54	3,700,000	7,600				
35 days	63	3,890,000	10,000				
39 days	66	4,180,000	9,300				
44 days	76	4,220,000	12,800				
51 days	79	4,400,000	13,600				
57 days	86	4,820,000	8,900				
73 days	90	5,780,000	12,600				
200 days	110	6,048,000	—				

* The data in this table are arranged in the same manner as in Table XXVII.

erythrocytes to only 5,420,000, and the return to normal occurred four days later. In a second experiment the same general results were obtained, but in less striking form. In this type of anaemia it is therefore also evident that absence of the spleen delays the regeneration of the blood; that is, interferes presumably with the normal reaction of the bone-marrow.

Repair of Blood in the Splenectomized Dog After Hemorrhage

A third method of study was to produce anæmia by hemorrhage and to observe the rapidity of repair in the splenectomized as contrasted with the normal animal (see Table XXX).

TABLE XXX*

REPAIR AFTER HÆMORRHAGIC ANÆMIA IN A SPLENECTOMIZED ANIMAL, WITH CONTROL

Dog 42. Weight 8,020 gm. (85 days after splenectomy)				Dog 55. Weight 7,860 gm. Normal		
100 c.c. of blood taken from a vein				150 c.c. of blood taken from a vein		
	Hæmo-globin per cent.	Red blood cells	Leucocytes	Hæmo-globin per cent.	Red blood cells	Leucocytes
Before	92	5,160,000	22,400	95	5,200,000	10,200
1 day	78	4,570,000	26,200	78	5,100,000	8,300
4 days	68	4,310,000	24,100	78	4,900,000	10,500
8 days	76	3,820,000	22,100	105	6,120,000	10,400
12 days	85	4,380,000	16,800	—	—	—
18 days	84	4,210,000	16,100	—	—	—
26 days	70	4,850,000	17,400	108	6,460,000	8,600
34 days	74	4,100,000	12,600			
40 days	90	4,480,000	10,200			
46 days	93	4,390,000	16,400			
54 days	95	4,760,000	13,100			
61 days	90	4,850,000	14,900			
67 days	102	5,100,000	19,200			

* The data in this table are arranged in the same manner as in Table XXVII

Here it is seen that a small loss of blood causes a markedly chronic anæmia in the splenectomized dog, and that repair is greatly delayed. After a loss of 100 c.c. of blood the hæmoglobin of the splenectomized dog was lowered on the fourth day to 68 per cent. and the erythrocytes to 4,310,000, with a return to normal sixty-three days later. From the control dog of slightly less weight, 150 c.c. of blood were taken, the hæmoglobin dropping to 78 per cent. and the red cells to 4,900,000 on the fourth day and promptly returning to normal by the eighth day.

Although we have not been especially interested in the changes in the white cells, these have been followed in some instances. In Table XXXI, which follows, the leucocytic counts of the animals presented in Table XXVII are shown.

TABLE XXXI *

LEUCOCYTE AND DIFFERENTIAL COUNTS AFTER HÆMOLYTIC SERUM

Dog 51. (26 days after splenectomy)					Dog 53. Normal				
	Leucocytes	Polymorphonuclear cells, per cent.	Small lymphocytes, per cent.	Eosinophils, per cent.		Leucocytes	Polymorphonuclear cells, per cent.	Small lymphocytes, per cent.	Eosinophils, per cent.
Before	24,200	86	9	3	Before	24,000	71	12	6
1 day	18,600				1 day	24,000	92	4	1
2 days	56,000				4 days	28,600			
3 days	28,400	73	8	12	8 days	36,000			
6 days	52,000				10 days	38,400	81	11	0
8 days	51,000	85	6	5	12 days	31,200	76	18	2
11 days	42,400	73	13	8	14 days	18,200	68	21	2
14 days	31,100	63	25	5	17 days	17,800	73	24	1
17 days	17,200				20 days	13,200	60	32	2
28 days	13,000	54	20	20	24 days	14,100	66	26	5
41 days	18,000	60	20	16	28 days	19,100			
51 days	16,800	54	20	17	31 days	11,900	61	28	5
62 days	18,000	65	17	14	44 days	16,100			
79 days	17,200	51	24	20	59 days	11,200			
93 days	16,400				66 days	25,000	78	15	5
100 days	12,200				72 days	22,400			
136 days	10,000	50	23	24	86 days	19,100	67	22	2
					95 days	12,800			
					107 days	10,200			

Normoblasts and polychromatophilia constantly present after injection of serum until 28th day. One normoblast seen in last count

Normoblasts present after injection of serum for 17 days, none thereafter. Polychromatophilia less marked than in dog 51

* The data in this table are arranged in the same manner as in Table XXVII.

It is evident that hæmolytic serum causes in the splenectomized dog a leucocytosis which runs higher than that in the normal dog. The increase in cells is chiefly in the polymorphonuclear leucocytes, though there is in both normal and splenectomized animals a moderate in-

crease in the lymphocytes and in the latter a persisting increase in the eosinophiles.

Another interesting point concerns the change in resistance of the red cells after administration of haemolytic serum. As has been shown in an earlier chapter, the increased resistance of the red blood-cells of the splenectomized animal to the action of hypotonic salt solution and to haemolytic serum is "not due to an increased anti-haemolytic power of the animal's serum or to a diminished complementary value of the serum, but is a property of the erythrocytes themselves." In the course of the present investigation it was noted that shortly after the administration of haemolytic serum the cells of the splenectomized animals, instead of showing a greater resistance to hypotonic solution, became less resistant, and this decreased resistance persisted in the splenectomized animal for a longer period than in the normal animal. This is shown in the following abridged table:

TABLE XXXII
RESISTANCE OF RED BLOOD-CORPUSCLES AFTER INJECTION OF HÆMOLYTIC SERUM *

Dog 51. (26 days after splenectomy)	Per cent. salt solution							
	0.25	0.3	0.35	0.4	0.45	0.5	0.55	0.6
Before serum was administered.....	+	+	P	P	O	O	O	O
3 hours after serum was administered.....	+	+	P	P	P	O	O	O
6 days after serum was administered.....	+	+	P	P	P	P	P	P
8 to 34 days after serum was administered.....	+	+	P	P	P	P	P	P
41 to 76 days after serum was administered.....	+	+	P	P	P	O	O	O
100 days after serum was administered.....	+	+	P	P	O	O	O	O
Dog 53. Normal	—	—	—	—	—	—	—	—
Before serum was administered.....	+	+	P	P	O	O	O	O
18 hours after serum was administered.....	+	+	P	P	P	P	P	O
8 to 12 days after serum was administered.....	+	+	P	P	P	P	P	P
20 to 95 days after serum was administered.....	+	+	P	P	P	O	O	O

* + indicates complete haemolysis; P, partial haemolysis; and O, no haemolysis.

Here it is seen that the erythrocytes of the splenectomized and the normal dog had the same resistance to salt solution before the injection, and likewise the same decrease in resistance eight days after the injection of haemolytic serum. The cells of the normal animal, however, returned to almost the original resistance after twenty days, while those of the splenectomized animal did not return to the same degree until the forty-first day. On the other hand, it will be seen that the splenectomized animal returned to the original point after one hundred days, while the normal animal failed to do so after ninety-five days.

In the anaemia produced by sodium oleate the decreased resistance, although it occurs, is not so striking in either the normal or the splenectomized animal as it is in the anaemia caused by haemolytic serum; the splenectomized animal, however, always shows a greater decrease in resistance than does the normal.

These observations may be summarized as follows:

In the splenectomized dog the anaemia caused by haemolytic poisons (haemolytic immune serum and sodium oleate) and by bleeding usually develops more gradually, is generally of a severer grade, runs a longer course, and is accompanied by a less rapid regeneration of the blood than is the case in the normal dog. Also, in the splenectomized dog, especially after the use of haemolytic serum, the leucocytosis is greater than in the normal animal.

The splenectomized dog almost uniformly exhibits an increased resistance of the red cells to hypotonic salt solution, but after the administration of haemolytic poisons, and especially haemolytic serum, this increased resistance disappears and a decreased resistance persists for varying

periods of time. The same change occurs in the normal dog, but in the latter the return to the previous or even increased degree of resistance is more rapid than in the splenectomized animal.

These results show that, although splenectomy leads to an increased resistance of the red blood-cells and to their slower destruction on the administration of a haemolytic agent—one factor in the lessened tendency to jaundice and to haemoglobinuria—yet there persists that same obscure disturbance which induces the anaemia occurring early after splenectomy, and which remains present, although latent for months, rendering any new haemolytic agent more effective and delaying the recovery from the anaemia which it causes.

It would appear, therefore, that of the phenomena associated with the absence of the spleen, two—the increased resistance of the red cells and the decreased tendency to jaundice after the administration of haemolytic poisons—are correlated, but that the anaemia itself is dependent upon some factor, as yet unknown, which operates in the absence of the spleen. After the administration of a haemolytic agent to a splenectomized animal this unknown factor dependent on the absence of the spleen prolongs the anaemia and retards repair, and the animal does not recover as quickly as does the normal animal. This, at the present stage of our knowledge, is the only explanation of the more severe and more prolonged anaemia occurring in splenectomized animals receiving haemolytic agents. Nevertheless, the increase in the red blood-cell count that we have found to follow the intraperitoneal injection of splenic extract suggests that the normal spleen exerts a stimulating effect on the bone-marrow, which naturally is lost after splenec-

tomy. It is probable that this loss may at least contribute to the retardation of repair in splenectomized animals, but the evidence on this point is insufficient to allow definite conclusions.

5. THE INFLUENCE OF THE SPLEEN UPON IRON METABOLISM

This investigation was undertaken to determine whether the tendency to anæmia in splenectomized dogs and the delayed regeneration of the blood, after the administration of haemolytic agents to such dogs, might be due in part to some influence of the spleen upon the iron metabolism, as has been claimed by Asher and his co-workers.

Our present knowledge concerning iron metabolism may be summarized as follows: Iron is absorbed to only a very limited extent from the gastro-intestinal tract, so that when abundant in the food it passes from the intestine for the most part unchanged. As much as is absorbed is taken up chiefly from the small intestine and carried by the lymph, to be deposited in the liver and to a lesser extent in the spleen, bone-marrow, and perhaps elsewhere, and this occurs whether the iron be in intimate organic combination, the so-called food iron, incapable of giving the characteristic microchemical reaction, or whether it be in the form of an organic or inorganic salt of iron. Moreover, from the work of Hausermann¹⁷⁰ and of Abderhalden,¹ it appears that, though iron salts are absorbed, the body is unable, or but very poorly able, to utilize them for the building of haemoglobin, being dependent for this constructive work upon the intimately combined food iron. On the other hand, iron salts are effective stimulants to the

blood-forming organs and conspicuously increase the utilization by them of the food iron.

The elimination of iron occurs almost wholly through the intestines, especially the colon, the quantity passing out in the urine constituting less than one per cent. of the total excretion in man and the dog. In the fasting dog the output found by Voit⁴⁵⁸ was 0.60 mgm. per kilo. of body weight per day, and on an adequate but iron-poor diet Gottlieb's¹⁵¹ dog excreted 0.34 mgm. For man the figures are lower. Cetti and Breithaupt,⁷⁰ while fasting, eliminated about 0.10 to 0.13 mgm. per kilo. per day, and in various studies on man 0.10 to 0.25 mgm. per kilo. per day have been found to be the intake required to maintain iron equilibrium. However, there is every reason to believe, as is suggested by the work of Schmidt,³⁹⁵ who fed mice for months on a diet extremely poor in iron, but obtained no fall in the haemoglobin, that the organism possesses great power of conserving its iron and of reutilizing it through some form of intermediary metabolism. When, however, Schmidt withdrew iron from the diet for several generations, the younger generations were extremely anaemic, and this anaemia disappeared upon restoring iron to the diet. As the iron-poor diet led to the disappearance of microchemically demonstrable iron from the liver, but affected to a much slighter degree that of the spleen, Schmidt concluded that the liver is the depot for iron from the food, and that the spleen, on the other hand, is the depot for iron from tissue and erythrocyte catabolism, and thus an important factor in the intermediary metabolism of iron.

If the spleen plays this part in iron metabolism, its absence might well interfere with the reutilization of iron

by the organism and lead to an increased iron elimination, and this Asher¹⁴ and his co-workers, Grossenbacher¹⁶ and Zimmermann¹⁹ claim to have demonstrated in dogs. They studied the iron elimination of four puppies from two litters; one from each litter was splenectomized and one from each kept as a control. The iron estimations were made at intervals of a few weeks, two months, and ten months after splenectomy, and in all their experiments they found an output much higher, often double, in the splenectomized animals as compared with the controls.

METHODS.—In our earlier experiments we studied the iron elimination during four-day periods, but found that such periods led to irregular results. In the work here reported, therefore, we present only observations based on periods of longer duration.

The animals were placed in metabolism cages with glass floors, and after they had been fed for several days on constant weighed amounts of the diet selected the rectum was emptied by the use of morphine; iron-free charcoal was added to the next feeding and the collection of fæces begun from the appearance of the charcoal; at the close of the period the rectum was again emptied with morphine, carmine added to the next feeding, and the fæces collected until carmine appeared in them. In the earlier experiments the urine also was analyzed, but as only traces of iron, less than one per cent. of the total elimination, were found the urine was omitted in our later analyses. To avoid the introduction of extraneous iron, the fæces were collected by means of a nickel spatula soon after being passed.

In one group of experiments representing the earlier periods after operation we have studied the output of iron

on the same dogs, both before and after splenectomy, without a change in diet. In another group, representing later periods, we have compared the output of normal control dogs with that of splenectomized dogs of approximately the same weight on corresponding diets.

The analyses were made by the method of Ripper and Schwarzer,³⁷⁶ slightly modified. The faeces collected for the entire period are placed in a quartz dish, dried, and ashed dry. The ash is extracted with boiling concentrated hydrochloric acid and filtered, and the residue washed with 20 per cent. hydrochloric acid. The residue and filter-paper are re-ashed and the extraction repeated. This ashing and extraction is continued until the extract ceases to give a positive test with KCNS.

The total filtrate is made up to a known volume and two duplicate portions, containing presumably 2 to 5 mgm. of Fe are taken. To each is added 1 c.c. of Merck's Blue label H₂O₂, and the solution evaporated to dryness on a water-bath. The residue is then redissolved in 1 c.c. of 20 per cent. HCl and 20 c.c. of boiling water used in four small portions, and then this washing with acid and water is repeated. In the course of this manipulation the entire solution is brought into a 200 c.c. Erlenmeyer flask.

All the specimens to be analyzed at any one time having been brought to this stage, a standard is prepared by placing in each of two 200-c.c. Erlenmeyer flasks 40 c.c. of a quantitative Fe₂Cl₆ solution containing about 0.002 gm. Fe. To each of the flasks, those containing the specimens and the two containing the standard, there are added in rapid succession 4 gms. of KI, the flasks immediately stoppered and placed in a water-bath at 60° C. for ten minutes. At the end of this time the flasks are removed

and to each 100 c.c. of cold water are immediately added and the flasks restoppered.

To each flask in turn is added starch solution and the contents titrated with sodium thiosulphate solution, approximately 1/250 N, until disappearance of the blue color, and then immediately titrated with weak iodine solution back to the first reappearance of the blue color. In each analysis the thiosulphate solution is freshly prepared and standardized against the two flasks of known Fe_2Cl_6 solution, and the iodine solution also is freshly prepared and standardized against the thiosulphate solution. The precision of the titration method is found to be greatly enhanced by the titration back with iodine to first reappearance of the blue color, and the calculation accordingly of the thiosulphate end point.

In control experiments performed by adding a known amount of iron to one of identical pairs of samples of ash of faeces an error of about 2 per cent. was observed.

The food used in these experiments consisted of casein, cracker-meal, lard, and fresh beef heart in proportions designed to give the desired amount of iron. The iron content of the food was determined by analyzing many large portions (each 50 gms. to 400 gms.) of the beef heart, cracker, and casein and obtaining average figures for use in calculating the iron content of the diets employed.

RESULTS.—In the accompanying tables are given in detail the final figures obtained in our studies.²² The experiments are divided into two groups: First, five animals on a constant diet were studied both before and for two weeks after splenectomy; these are arranged in Table XXXIII according to the iron content of the diet. Second, a group of five animals (Table XXXIV), two of which

served as normal controls and three for purposes of study at longer periods after splenectomy than are represented by the group in Table XXXIII. These five dogs were of about the same weight and were on diets of the same general character, but varying in the content of iron.

TABLE XXXIII
ELIMINATION OF IRON BEFORE AND AFTER SPLENECTOMY

Dog No.	Average weight	Duration of periods	Intake *	Output*		Time after splenectomy
				Before splenectomy	After splenectomy	
88	7,000	10 days	0.27	0.67	0.70	4-14 days
30	5,340	9 days	0.30	0.36	0.55	1-10 days
35	7,720	9 days	0.64	0.87	0.81	1-10 days
44	9,000	9 days	1.57	1.89	2.10	1-10 days
79	9,000	9 days	1.71	1.88	2.21	6-15 days

* Figures represent milligrammes of iron per kilo. per day.

TABLE XXXIV
ELIMINATION OF IRON IN NORMAL AND SPLENECTOMIZED DOGS
Normal

Dog No.	Controls				Time after splenectomy
	Average weight	Duration of period	Intake *	Output *	
79	9,000	9 days	1.00	1.42	
44	9,000	9 days	1.59	1.89	
79	9,000	9 days	1.71	1.88	

<i>Splenectomized</i>					
83	8,400	10 days	1.42	1.39	27-37 days
9	8,800	9 days	1.35	1.56	9 months
51	10,000	9 days	1.32	1.42	20 months

* Figures represent milligrammes of iron per kilo. per day.

Inspection of Table XXXIII shows that the iron outputs of Dogs 88 and 35 was unchanged by splenectomy, but that Dogs 30, 44 and 79 showed some increase. On the other hand, in Table XXXIV it will be seen that all three splenectomized dogs exhibited an output of iron as com-

pared with the intake closely comparable with that of the controls. From these studies it would appear, therefore, that during the first two weeks after splenectomy some, but not all, dogs show a slight increase in the output of iron, but that at one month, nine months, and twenty months after splenectomy we find no indication of such increased iron output. The occasional evanescent and inconstant increase in elimination of iron does not justify the conclusion that the spleen exerts an important influence on iron metabolism. Our results are obviously different from those of Asher and his associates, and as a possible explanation of this we would call attention to the extreme shortness of the periods—one to three days—employed by Asher and Grossenbacher, and to their failure to mark in any way the stools. In the studies of output ten months after splenectomy, as given by Asher and Zimmermann, the splenectomized dog in most of the experiments was much larger than the control, so that if the iron output of their dogs be calculated per kilo. of body weight it will be found that the output of the splenectomized animals approaches very closely that of the normal controls and is in some instances identical. It seems possible that in these studies ten months after splenectomy the apparent increase in iron output of the splenectomized animals was due rather to the size of the animals than to the splenectomy, and it is doubtful, therefore, whether the conclusions of Asher and Zimmermann, based on these experiments, are justified.

It is, however, difficult to explain the slight increase in the elimination of iron in three of five of our dogs during a period of two weeks following splenectomy. In our discussion ²² of this first study of iron metabolism we stated that possibly the occasional increased output of iron might

have some relation to the anaemia which occurs in the early weeks after splenectomy and which varies in degree in different animals. Later investigations offer some support to this hypothesis. In a recent study¹⁴⁹ in which four dogs were used in an investigation of nitrogen metabolism before and after splenectomy the elimination of iron in the faeces was determined. The methods used differed from the earlier study in that in all animals the iron elimination was determined before splenectomy and during one to three periods after splenectomy, and also in that Neumann's method³¹⁵ of determining iron was used instead of the Ripper-Schwarzer method. The periods of study varied from five to eight days and represented intervals of three days to three months after splenectomy. As the dogs were kept in all periods on the same diet, in both quantity and quality, the iron intake was not determined. The results are presented in Table XXXV.

TABLE XXXV *

IRON ELIMINATION BEFORE AND AFTER SPLENECTOMY

Dog No.	Before splenectomy		After splenectomy		
	Period I	Period II †	Period III †	Period IV †	
48	17.6	17.9	16.8		
52	10.5	10.4	9.8		
56	7.4	9.0	18.4		
57	10.9	10.8			9.9

* The figures in this table represent milligrammes of iron per day in faeces.

† The periods after splenectomy were 14 and 55 days for dog 48; 13, 41, and 70 days for dog 52; 10 and 86 days for dog 56, and 3 days for dog 57.

In three of four dogs no important change in the elimination of iron occurred after splenectomy. In the fourth (Dog 56) there was an increase of 1.6 mg. per day during the period (ten days) immediately after splenectomy, amounting to an increase of 21.6 per cent. over the fore-

period. In the final period three months after splenectomy the output showed an increase of 148 per cent. over the fore-period and double that of Period II. The intake of iron was not determined, but, since the food intake was constant throughout all the periods, we have reason to believe that this was a constant factor. Of the four animals, two showed no anæmia and a third only a slight reduction in haemoglobin and red cells. The fourth dog (No. 56) showed a relatively severe anæmia (see Table LIII in section devoted to metabolism). As this last dog was the only one to show any unusual elimination of iron after splenectomy, the question naturally arises: Is the increased elimination of iron due to the anæmia or to the absence of the spleen? We incline to the former view and conclude that the spleen exerts no constant and important influence upon iron metabolism.*

* One other attempt to elucidate the iron problem has been the feeding of an inorganic salt of iron before and after splenectomy. Such experiments in the normal animal are, as a rule, of little value, as iron so administered is not absorbed to any appreciable extent, but is eliminated almost completely.¹⁶⁴ It seemed worth while, however, to see if any change in absorption occurred in the absence of the spleen. Two dogs on the usual constant diet were, therefore, fed daily 55 mgm. of iron as ferrous sulphate during a period of one week before and a like period after splenectomy. In each instance the second period of analysis began four days after splenectomy. No essential or constant difference was found in the elimination of the two periods. In one animal, of 55 mgms. of iron given daily, 52 mgm. were eliminated daily before and 50.5 after splenectomy; in the other animal the figures were 51.2 before and 52.5 after splenectomy: essentially negative results.

CHAPTER V

CONTROL EXPERIMENTS: THE DIVERSION OF THE SPLENIC BLOOD FROM THE LIVER WITHOUT REMOVAL OF THE SPLEEN

(1) BY LIGATION OF THE SPLENIC VEIN, (2) BY TRANSPLANTATION OF THE SPLENIC VEIN INTO THE VENA CAVA, (3) BY ECK FISTULA.

HITHERTO in all our discussions of the phenomena following splenectomy—the anaemia, the increased resistance of the red cells, and the decreased tendency to jaundice—we have assumed that, inasmuch as the spleen was absent, the changes described are probably due to the loss of some function peculiar to that organ.

As to the exact nature of the function lost, no definite opinion has thus far been given. Three possibilities, however, readily suggest themselves: (1) The loss of some function of blood destruction or regeneration resident in the spleen itself; (2) the loss of an internal secretion acting on the distant haemopoietic tissues, as the bone-marrow, and (3) the obliteration of the venous drainage of the spleen, which, in that it is an important source of the portal blood, may have an essential relation to some peculiar function of the liver. It is obvious that the value of some of these hypotheses might be tested without removal of the spleen by diverting its venous outflow from the liver. We have therefore repeated²²⁹ many of our experiments, but, instead of removing the spleen, the splenic vein has either been ligated or transplanted in the vena cava, or an Eck fistula has been established. So far as we know, exactly similar experiments have not previously been at-

tempted, though two observations have a bearing on the problem. Nassau³¹³ found that the number, color, and resistance of the red cells remained unchanged after simple Eck fistula, but, as he gives only one count before and one after the operation, his observations are not conclusive. The other report which concerns us is Pribram's³⁶¹ artificial constriction of the splenic vein with resultant passive congestion of the spleen. The slight anæmia that this caused is analogous to some of the results we report below.

If the anæmia following splenectomy is caused by the removal with the spleen of some necessary factor in blood formation or of a hormone essential to the hæmopoietic tissues, this factor should not operate in the vein transplantation and Eck fistula experiments unless it is also essential that such a substance have direct approach to the liver for its proper functioning or activation. If, on the other hand, the anæmia is largely due to the interference with the supply of splenic blood to the liver, it should occur in all the animals. These problems and also that concerning the influence of the experimental procedure on increased resistance of the red cells will be considered first, and then problems such as the decreased tendency to jaundice and the prolonged repair of anæmia in the splenectomized animal when hæmolytic agents are given.

METHODS.—In the ligation experiments it was found necessary, on account of the numerous branches and the anastomoses of the splenic vein with veins from the stomach, to tie all branches of the splenic vein shortly after they left the spleen. In the earlier operations one of the subdivisions of the artery was also ligated, to lessen the supposed danger of rupture. This, later, was abandoned

when it was found to be unnecessary as well as undesirable on account of infarct production. Post-mortem examination of animals subjected to this operation showed in each case that all branches had been tied, that the vessel beyond the ligation was much diminished in size or completely obliterated, and that there was usually little attempt at compensatory development of new veins from the adherent omentum. When such new-formed veins were present it was found that seldom did they empty into the portal system and therefore, as a rule, did not complicate the experiment.

Eck fistula was performed in the usual manner, the proximal end of the portal vein being tied off above its new anastomosis with the vena cava just before its entrance into the liver. Transplantation of the splenic vein into the inferior vena cava presented considerable difficulty on account of the smallness of the vein and the necessity of stretching it a little to make it reach the vena cava. In only one experiment, however, was it found that the transplanted vein had been occluded by thrombus. The success of the operation was always determined by examination of the vessels at autopsy, and in some instances injection specimens were prepared and dissected to make doubly sure that no new anastomoses had formed.

The particular phenomena studied were (1) the quantitative changes in counts of the red blood-cells, leucocytes, and haemoglobin; (2) the resistance of the red cells to hypotonic salt solution; (3) the general condition of the animals as indicated by their weight and condition of the urine, and (4) the gross and microscopical appearance of the organs at autopsy.

The dogs were kept on the usual mixed diet of "table scraps," which has been shown to maintain properly normal

dogs as well as those convalescing from operations other than splenectomy without the development of anaemia.³³⁷

Our studies have been made on twelve dogs, grouped as follows: Ligation of splenic vein, 4; transplantation of splenic vein, 2; Eck fistula, 2; and as controls: Splenectomy, 3; transplantation of the inferior mesenteric vein, 1. In the case of the last dog it was intended to transplant the splenic vein, and it was not until autopsy that we found that the inferior mesenteric vein had been used by mistake.

CHANGES IN THE SPLEEN

In those dogs in which all branches of the splenic vein had been ligated, and in which adequate new venous channels had failed to develop, the spleen showed considerable change. Before the operation was completed the spleen had increased to almost double its size and taken on a dark-purple color. If the animal was allowed to survive two or more months the spleen, at autopsy, was found to be considerably smaller and much firmer than normal. The capsule was slightly thickened and puckered, and the organ had a pale-bluish color. On section the tissue cut with increased resistance. A few small shrunken infarcts were occasionally found. The cut surface was less bloody than usual and showed an increase of fibrous tissue, with indistinct Malpighian corpuscles. Histologically, the tissue appeared to be condensed, with collapse of the sinuses, rather than to exhibit true fibrous hyperplasia. Some haemosiderin pigment was found, the Malpignian corpuscles were small, and here and there were small areas of hyaline degeneration which did not respond to Lugol's test. Thromboses were not found except in connection

with old post-operative infarcts. In the earlier experiments in which a branch of the artery was also ligated a bulging hemorrhagic infarct appeared in the corresponding area of the spleen. The neighboring lymph-nodes and the liver were apparently unchanged. Speculation as to the adjustment of the splenic circulation in these cases must remain unsatisfied. The most probable explanation seems to be that the elastic spleen is able not only to accommodate the increasing pressure without rupture, but, with the aid of the minute capillaries in the adherent omentum, to maintain sufficient degree of nutrition to prevent necrosis. We have seen, however, that the exchange is not sufficient to prevent atrophy.

At the time of the operation for Eck fistula and the splenic vein-vena cava anastomosis there is necessarily a temporary occlusion of the large veins. The spleen and intestines become very dark and turgid, but when the clamps are removed these organs return quickly to normal color. In animals so treated no changes are found at autopsy except adhesions and perisplenitis.

CHANGES IN THE BLOOD

An example of the changes in the blood which occur after ligation of the splenic vein is given in Table XXXVI.

It may be seen that this animal developed a moderate degree of anaemia lasting several weeks and similar to, but less severe than, that following splenectomy. The anaemia is accompanied by a temporary slight increase in resistance of the red cells to hypotonic salt solution. As in splenectomy, there is an immediate leucocytosis, due to polymorphonuclear and transitional forms, but prolonged by a more persistent lymphocytosis and eosinophilia.

A very slight drop in weight occurs after operation, but later there is a distinct increase above the original weight. It has been shown elsewhere,³³⁷ by the study of control operations such as nephrectomy, that the results here described are not merely post-operative. Other dogs with ligated veins gave similar results, as, for example,

TABLE XXXVI
BLOOD CHANGES FOLLOWING LIGATION OF SPLENIC VEINS

Dog 47	Hæmoglobin	Red blood cells per cmm.	Hæmolysis *		Leucocytes per cmm.	Polymorpho-nuclears	Small lymphocytes	Large transitionalis	Eosinophils	Weight
	per cent.		Begins	Complete						
Before	102	6,275,000	0.475	0.35	9,800	7,200	700	1,800	100	6.0
1 day after	98	6,110,000			19,000	15,200	950	2,850	0	5.7
4 days after	92	5,350,000	0.475	0.275	16,500					5.6
1 week after	90	5,520,000	0.425	0.275	16,800	13,600	1,300	1,600	300	5.4
2 weeks after	72	4,900,000	0.425	0.3	18,800	15,400	1,900	1,500	0	
3 weeks after	70	4,600,000	0.45	0.3	21,000	16,800	3,100	300	300	
4 weeks after	78	5,000,000			18,600					6.4
5 weeks after	74	4,380,000			15,000					
7 weeks after	80	5,040,000	0.475	0.275	12,800	10,000	1,700	1,100	0	7.3
10 weeks after	82	5,050,000	0.45	0.3	12,400					
12 weeks after	95	5,830,000	0.475	0.325	14,800	9,500	3,400	1,500	400	7.5
13 weeks after	92	6,410,000	0.475	0.35	14,900	9,200	3,600	1,200	900	
14 weeks after	95	6,150,000			15,600	12,200	2,500	600	100	7.4

* The figures in these columns refer to the strongest percentages of salt solution in which haemolysis was first noticed and in which complete haemolysis first occurred.

Dog 51 with a maximum drop of 14 per cent. of haemoglobin and 1,200,000 red cells, and Dog 74 with a haemoglobin loss of 24 per cent. and in red blood-cells of 1,800,000.

The effect of diverting the splenic venous blood from the liver by transplanting the splenic vein into the vena cava is shown in Table XXXVII.

Many of the same changes as after ligation are found

here. In another dog (No. 71) similarly treated the increased resistance was much more marked; before operation, haemolysis began at 0.50 and was complete at 0.35; soon after operation it began at 0.425 and was complete at 0.25. Anæmia was also present, as shown by a drop of 20 per cent. in haemoglobin and of 1,800,000 red blood-cells. A third animal (16) lost 35 per cent. haemoglobin

TABLE XXXVII

BLOOD CHANGES FOLLOWING ANASTOMOSIS OF THE SPLENIC VEIN WITH THE VENA CAVA

Dog 4	Haemoglobin per cent.	Red blood cells per cmm.	Hæmalysis *		Leucocytes per cmm.	Polymorpho-nucleus	Small lymphocytes	Large transitionalis	Eosinophilia	Weight kilos.
			Begins	Complete						
Before	97	6,960,000	0.45	0.35	16,100	8,400	4,700	1,600	1,400	9.4
1 day after	104	7,040,000	0.425	0.3	34,000	29,600	3,100	1,300	0	
1 week after	75	5,550,000	0.45	0.3	19,300	12,400	3,700	1,900	1,300	9.7
2 weeks after	68	5,020,000	0.475	0.275	16,900	11,800	2,700	2,200	200	9.4
3 weeks after	70	4,530,000								9.6
4 weeks after	80	4,510,000	0.475	0.3	14,700	9,600	2,800	1,500	800	10.4
5 weeks after	74	5,440,000	0.475	0.325	14,000	9,800	2,800	420	1,080	10.5
6 weeks after	78	5,630,000	0.45	0.3	11,200	6,300	4,000	560	360	
7 weeks after	82	5,770,000	0.45	0.325	11,100	7,400	2,900	200	600	12.9
8 weeks after	85	6,060,000	0.45	0.325						
9 weeks after	96	6,500,000								13.2

* The figures in these columns refer to the strongest percentages of salt solution in which haemolysis was first noticed and in which complete haemolysis first occurred.

and over 2,000,000 erythrocytes, and a fourth, 22 per cent. haemoglobin and over 1,000,000 erythrocytes.

The changes in an Eck fistula experiment are seen in Table XXXVIII.

In another Eck fistula dog (No. 68) the increased resistance of the red cells was more marked (before operation, haemolysis began at 0.45 and was complete at 0.3; soon after operation it began at 0.425 and was complete

at 0.225), and the animal lost 33 per cent. haemoglobin and 2,000,000 red blood-cells in four weeks' time after operation. In a third (No. 5) with an even greater drop, the blood picture was complicated by the occurrence of infection, for which reason the figures are not given.

That interference with the portal circulation inflow from organs other than the spleen may cause changes in

TABLE XXXVIII
BLOOD CHANGES FOLLOWING ECK FISTULA

Dog 31	Hæmoglobin	Red blood cells per cmm.	Hæmolysis *		Leucocytes per cmm.	Polymorpho-nuclears	Small lymphocytes	Large transitionalis	Eosinophils	Weight kilos.
	per cent.		Begins	Complete						
Before	99	6,500,000	0.45	0.325	13,200	9,800	2,700	500	200	16.1
1 day after	88	6,040,000	0.475	0.325	36,000	32,400	2,800	800	0	
3 days after	84	6,300,000	0.45	0.325	32,400	27,500	2,900	1,600	400	14.2
5 days after	72	5,400,000	0.425	0.3	24,000	17,300	3,800	2,400	500	14.2
1 week after	74	5,500,000	0.425	0.3	18,800	13,700	3,400	800	900	13.6
2 weeks after	68	5,040,000	0.425	0.275	19,600	15,500	3,900	600	600	13.5
3 weeks after	73	5,110,000	0.425	0.275						
4 weeks after	67	4,460,000	0.425	0.3	21,000	15,500	4,400	600	500	14.6
5 weeks after	72	4,880,000	0.475	0.325	18,400	12,900	3,700	600	1,200	14.2
6 weeks after	84	5,600,000	0.475	0.325	16,000	10,500	3,800	400	1,300	14.9
7 weeks after	90	6,200,000	0.45	0.3	15,200	10,300	2,800	600	1,500	15.3
8 weeks after	98	6,520,000	0.45	0.3	15,800	11,400	3,500	300	600	15.4

* The figures in these columns indicate the percentages of salt solution in which haemolysis was first noticed and in which haemolysis first became complete.

the blood is shown in another experiment (see Table XXXIX) in which the inferior mesenteric vein was anastomosed with the vena cava.

This dog developed an anaemia of mild grade and recovered from it sooner than did the other dogs of this series. Although little weight can be placed on a single experiment such as this, the observation tends to support the theory that the supply of portal blood to the liver is a

factor in the production of the changes we have observed to occur after splenectomy.

From these observations the following conclusions may be drawn:

1. In dogs in which the splenic vein has been ligated or transplanted into the inferior vena cava, or in which an Eck fistula has been made, an anaemia occurs which re-

TABLE XXXIX

BLOOD CHANGES FOLLOWING TRANSPLANTATION OF INFERIOR MESENTERIC VEIN. CONTROL EXPERIMENT

Dog 55	Hæmo-globin <i>per cent.</i>	Red blood cells per cmm.	Hæmolysis *		Weight <i>kilos.</i>
			Begins	Complete	
Before.....	100	5,940,000	0.45	0.3	14.7
2 days after.....	76	5,240,000	0.4	0.25	
3 days after.....	75	5,100,000	0.4	0.25	13.9
10 days after.....	76	4,880,000	0.45	0.275	
2 weeks after.....	85	5,400,000	0.425	0.25	
3 weeks after.....	84	5,890,000	0.425	0.25	
4 weeks after.....	78	5,600,000			14.7
5 weeks after.....	88	5,720,000			
6 weeks after.....	92	5,800,000	0.425	0.275	15.3

* The figures in these columns indicate the percentages of salt solution in which hæmolysis was first noticed and in which hæmolysis first became complete.

sembles that following splenectomy and shows the same general variations in degree and duration.

2. The resistance of the red cells to hypotonic salt solution is quickly increased, sometimes coincident with and sometimes preceding the anaemia. It gradually returns to normal in about the same length of time as it takes the anaemia to disappear, differing in this particular from the results after splenectomy.

3. There is an initial leucocytosis, involving at first the polymorphonuclear leucocytes and transitional cells. As the total leucocytosis diminishes there is both a relative

and actual increase of small lymphocytes and usually also of eosinophiles. This may either be temporary or last during the rest of the period of observation, and thus differs from the ordinary postoperative leucocytosis.

4. Ligation of the splenic vein is followed by considerable atrophy of the spleen, but not by necrosis or thrombosis. There is rarely adequate new vein formation. The other operations cause little or no change in the spleen.

5. Whether the disturbances described are due to the loss of a certain volume of blood to the liver, or, as has been previously suggested, to the loss of a splenic hormone acting on the hæmopoietic tissues, it is impossible to say. If due to the former, the method of production of the anæmia still remains unexplained. It is evident, also, that the latter theory has no value unless it is assumed, also, that the supposititious hormone normally is activated by passage through the liver.

RESISTANCE TO HÆMOLYTIC AGENTS

In the previous pages has been discussed the effect upon the blood picture of three methods (transplantation of the splenic vein into the vena cava, Eck fistula, and ligation of the splenic vein) of diverting the splenic blood from the liver. It now remains to present the results of a study of the influence of these procedures on the action of hæmolytic agents. Do they bring about a decreased tendency to jaundice when a hæmolytic agent is given and retard the repair of an artificially produced anæmia, as is the case in splenectomized animals?

As hæmolytic agents we have employed both toluylenediamine and hæmolytic immune serum. The hæmolytic serum was prepared by injecting, at regular intervals, the

erythrocytes of the dog into rabbits. All injections of serum so prepared were made intravenously into the dog. Merck's meta-toluylenediamine was given by stomach-tube, and in adequate doses never failed to cause anaemia and jaundice. In each experiment the control animal received the same proportionate amount of drug or serum per kilo. of body weight as did the test animal. Dogs of approximately the same size were selected, and, on account of the occasional necessity of catheterization, female dogs were used whenever available. Splenectomized as well as normal animals were included in the series to further comparison with our previous results. Daily and, later, weekly examinations of urine and complete blood examinations (including resistance of erythrocytes to hypotonic salt solution) were made and records of weight were kept. Each animal was studied as to condition of urine and blood before the experiment. Both cage urine and catheterized

TABLE XL

JAUNDICE AFTER ADMINISTRATION OF TOLUYLENE DIAMIN (0.34 GM. PER KILO.)

Time after administration	Dog 71 Splenic vein transplant (2 mos.)	Dog 79 Splenectomy (1½ mos.)	Dog 51 Ligation (2 mos.)	Dog 75 Normal control
	0.425 B. H.- 0.325 C. H.* No anaemia	0.40 B. H.- 0.275 C. H. No anaemia	0.45 B. H.- 0.275 C. H.; Slight anaemia	0.425 B. H.- 0.275 C. H. No anaemia
Before	Urine normal	Urine normal	Urine normal	Urine normal
1 day after	Bile trace	Bile slight	Bile moderate	Bile marked
3 days after	Bile heavy trace	Bile slight	Bile moderate	Bile marked
5 days after	Bile trace	Bile moderate	Bile moderate	Bile marked
7 days after	Bile absent	Bile moderate	Bile moderate	Bile marked
9 days after	Bile absent	Bile moderate	Bile moderate	Bile moderate
11 days after	Bile absent	Bile trace	Bile faint trace	Bile trace
13 days after	Bile absent	Bile trace	Bile absent	Bile trace
15 days after	Bile absent	Bile trace	Bile absent	Bile trace
17 days after	Bile absent	Bile absent	Bile absent	Bile absent

* This space in this and following tables refers to the condition of the blood before beginning the experiment. The figures indicate strength of salt solution at which haemolysis occurs; B. H. indicates beginning haemolysis, C. H. complete haemolysis. Increasing amounts of bile in the urine are expressed in the following terms: faint trace, trace, heavy trace, slight, moderate, marked.

specimens (in doubtful cases) were examined, and the presence of bile-pigment in the urine, determined by Gmelin's and Rosenbach's tests, was taken as the surest evidence of jaundice. At the termination of the experiment the animals were sacrificed in order to determine the exact anatomical disturbance caused by the operation.

As may be seen in Table XL, in all animals receiving toluylenediamine, control as well as experimental, some bile appeared in the urine. The bile was less in amount, however, and lasted for shorter periods in the test animals than in the normal controls. In fact, animals with the vein transplant and Eck fistula showed even less tendency to jaundice than the splenectomized animal.

TABLE XLI
JAUNDICE AFTER ADMINISTRATION OF TOLUYLENEDIAMIN (0.13 GM. PER KILO.)

Time after administration	Dog 68 Eck fistula (1 mo.)	Dog 55 Mesenteric vein transplant (1½ mos.)	Dog 77 Normal control No anæmia
	0.4 B. H.-0.275 C. H. Anæmia present	0.425 B. H.-0.275 C. H. No anæmia	0.425 B. H.-0.35 C. H.
Before	Urine normal	Urine normal	Urine normal
1 day after	Bile absent	Bile trace	Bile trace
2 days after	Bile faint trace	Bile trace	Bile marked
3 days after	Bile faint trace	Bile moderate	Bile moderate
5 days after	Bile absent	Bile doubtful	Bile moderate
7 days after	Bile absent	Bile absent	Bile trace
9 days after	Bile absent	Bile absent	Bile absent

Another experiment (see Table XLI) in which a smaller single dose was administered gave similar results. It was in this experiment that the animal that was supposed to have had the splenic vein transplanted was found at autopsy to have a branch of the inferior mesenteric vein transplanted by mistake, thus unwittingly causing an excellent double control for the Eck fistula test (No. 68). The increased resistance to jaundice of this dog (No. 55) as

compared to the normal (No. 77) is still further evidence of the importance of the mechanical factor of blood supply to the liver.

In a third experiment in which three smaller repeated doses of toluylenediamine were given to animals with splenic vein transplant and splenic vein ligation and to a normal control these results were confirmed. The vein transplant dog, though his red cells at the time were the least resistant of the three, failed to develop jaundice; the other two did. The jaundice in the ligation experiment, however, lasted three days, and that of the control nine days.

In the experiments with haemolytic immune serum the same results were obtained. In a preliminary experiment

TABLE XLII
JAUNDICE AFTER ADMINISTRATION OF HÆMOLYTIC SERUM (SERUM NO. 3, 0.4 C.C.
PER KILO.)

Time after administration	Dog 4 Vein switch (2 mos.)	Dog 2 Splenectomy (2½ mos.)	Dog 25 Normal control
	0.45 B. H.-0.325 C. H. Slight anaemia	0.425 B. H.-0.25 C. H. Slight anaemia	0.475 B. H.-0.35 C. H. No anaemia
Before	Urine normal	Urine normal	Urine normal
1 day after	No bile	Bile moderate	Bile trace
2 days after	No bile	Bile moderate	Bile moderate
4 days after	No bile	Bile moderate	Bile absent
6 days after	No bile	Bile absent	

with three animals, in which large doses of serum were given, the Eck fistula dog alone survived and developed jaundice; one with vein transplant and also a normal control died in a few hours; the control, however, had already developed haemoglobinuria, whereas the vein transplant dog failed to develop either haemoglobinuria or jaundice.

With a weaker serum (see Table XLII) the greater resistance of the vein transplant animal is shown. A result

contrary to our former experiences was obtained in this experiment, in that No. 2 (splenectomized) developed as severe a jaundice as did the control.

In another experiment (see Table XLIII) the same instructive results were obtained with a serum of less haemolytic power.

TABLE XLIII
JAUNDICE AFTER ADMINISTRATION OF HÆMOLYTIC SERUM (SERUM NO. 3, 0.2 C.C.
PER KILO.)

Time after administration	Dog 16 Vein transplant (occluded vessels) (1 mo.)	Dog 1 Splenectomy (3 mos.)	Dog 51 Ligation (6 mos.)	Dog 27 Normal control
	0.4 B. H.- 0.25 C. H. Anæmia present	0.4 B. H.- 0.25 C. H. Slight anæmia	0.45 B. H.- 0.325 C. H. No anæmia	0.45 B. H.- 0.35 C. H. No anæmia
Before	Urine normal	Urine normal	Urine normal	Urine normal
1 day after	No bile	No bile	No bile	Bile marked
2 days after	No bile	No bile	Bile marked	Bile moderate
3 days after	No bile	No bile	Bile marked	Bile trace
5 days after	No bile	No bile	Bile marked (Bile still present on the 14th day)	Bile absent

As the transplanted splenic vein of No. 16 was found at autopsy to have been occluded by a comparatively recent thrombus (probably antedating the administration of serum), this experiment must be regarded as analogous to a ligation experiment. The relatively slight tendency to jaundice in this animal as compared with No. 51 may be largely explained by the greater resistance of the red cells in the former animal.

The results of these experiments, while somewhat discordant, indicate that the mechanical factor of the method of the blood's approach to the liver is of importance in determining the degree of jaundice after the administration

of haemolytic agents. Our results in this regard are in accord with those obtained in our comparative studies of the effect of injecting haemoglobin into the portal as contrasted with the general circulation (see page 64).

The interpretation of the severity and duration of the anaemia caused in the various test animals is complicated by several factors. In the first place, the original operation necessary to produce the venous anastomosis has been shown to cause anaemia. As the test animals were sometimes given the haemolytic agent while more or less anaemic, we have had to analyze our results with constant reference to this factor. Moreover, we have found that in most cases the severity of the anaemia largely parallels the resistance of the red cells. In some cases, however, animals with the most fragile cells develop the least anaemia after administration of haemolytic agents. Changes in weight in the animals of these experiments seem to bear no definite significant relation to the anaemia.

We have previously stated²²⁷ that "the anaemia caused in splenectomized animals by haemolytic agents is, as shown by direct blood examination, of greater severity, runs a longer course, and has a longer period of repair." By greater severity was meant that the haemoglobin and red-cell count reached lower figures than in the control. The actual blood destruction, however (if determined by estimating the change from the condition immediately before administering haemolytic agents), was usually less in splenectomized than in normal dogs. Our experiments of this year show that the same statements hold true after transplantation of the splenic vein into the vena cava, Eck fistula, and ligation of the splenic veins.

The actual blood destruction of the test animals has

been constantly less than in the normal controls, and in a few instances even the degree of anæmia as expressed by the haemoglobin and red-cell count was less severe in the test animals. As a constant feature, the greater severity of the anæmia must therefore be considered as open to

TABLE XLIV
DURATION AND SEVERITY OF ANÆMIA AFTER ADMINISTRATION OF TOLUYLENE-DIAMIN (0.34 GM. PER KILO.)*

Time after administration	Dog 71 Splenic vein transplant			Dog 79 Splenectomy		
	Hæmo-globin	Red blood cells	Resistance	Hæmo-globin	Red blood cells	Resistance
	per cent.		B. H. C. H.	per cent.		B. H. C. H.
Before	98	6,785,000	0.45 -0.325	74	5,720,000	0.4 -0.275
1 day after	102	6,940,000	0.5 -0.3	84	4,770,000	0.5 -0.275
3 days after	58	5,490,000	0.475-0.275	60	4,250,000	0.425-0.275
5 days after	68	5,340,000	0.475-0.325	43	3,380,000	0.45 -0.25
7 days after	72	4,820,000	0.475-0.325	52	3,820,000	0.45 -0.25
9 days after	83	5,070,000	0.5 -0.3	63	4,080,000	0.425-0.25
12 days after	96	5,890,000	0.5 -0.3	66	4,320,000	0.425-0.25
16 days after	98	6,210,000		69	4,530,000	
22 days after	96	6,350,000	0.5 -0.3	68	4,510,000	0.4 -0.275
29 days after				76	5,280,000	0.4 -0.25
35 days after			0.5 -0.3	88	5,590,000	0.4 -0.275
41 days after			0.475-0.325	88	5,480,000	0.4 -0.35
49 days after	98	6,800,000		92	5,680,000	0.4 -0.25

Time after administration	Dog 51 Ligation			Dog 75 Normal control		
	Hæmo-globin	Red blood cells	Resistance	Hæmo-globin	Red blood cells	Resistance
	per cent.		B. H. C. H.	per cent.		B. H. C. H.
Before	80	4,920,000	0.45 -0.275	85	6,180,000	0.425-0.275
1 day after	90	4,800,000	0.5 -0.325	96	5,080,000	0.45 -0.35
3 days after	60	3,820,000	0.375-0.25	48	4,590,000	0.475-0.275
5 days after				38	2,980,000	0.45 -0.3
7 days after				45	3,630,000	0.475-0.3
9 days after	62	3,310,000	0.45 -0.3	55	3,930,000	0.45 -0.3
12 days after	65	3,980,000	0.45 -0.275	68	4,800,000	0.475-0.3
16 days after	72	4,320,000		80	5,200,000	0.475-0.3
22 days after	74	4,650,000		85	5,820,000	0.425-0.3
29 days after	76	4,950,000	0.475-0.35			
35 days after	84	5,330,000				
41 days after	78	5,340,000	0.425-0.3			
49 days after	87	5,550,000	0.45 -0.3			

* The figures in the columns "Resistance" indicate the percentages of salt solution at which haemolysis was first noticed and first became complete.

question, while the actual blood destruction in the test animals is undoubtedly less than in the normal controls.

The duration of the anaemia of the test animals has been longer than that of the normal controls and paralleled that of the splenectomy controls. The difference, however, of both test and splenectomy dogs from the normal controls has been less striking than in our previous work, and, like the variable degree of anaemia after splenectomy, must be referred to variations in unknown factors (possibly such as diet, or differences in toxicity of the serum).

An experiment with toluylenediamine showing the character of the blood repair after various forms of experimental disturbance of splenic function is seen in Table XLIV.

TABLE XLV

DURATION AND SEVERITY OF ANAEMIA AFTER ADMINISTRATION OF HÆMOLYTIC SERUM (0.4 CC. PER KILO.)

Time after administration	Dog 4 Vein transplant (2 mos.)		Dog 2 Splenectomy (3½ mos.)		Dog 25 Normal control	
	Hæmo-globin	Red blood cells	Hæmo-globin	Red blood cells	Hæmo-globin	Red blood cells
	per cent.		per cent.		per cent.	
Before	97	6,960,000	85	4,950,000	102	6,400,000
1 day after	83	4,840,000	78	4,920,000	82	5,040,000
6 days after	65	3,940,000	55	3,670,000	58	3,950,000
10 days after	62	3,750,000	67	3,890,000	78	4,480,000
16 days after	70	4,390,000	67	4,090,000	80	4,880,000
19 days after	90	5,060,000			93	5,490,000
26 days after	96	6,010,000	78	5,260,000	106	6,060,000
31 days after	102	6,400,000	82	5,590,000		

In a second experiment the red cells of an Eck fistula animal dropped one million less than the control, but took two months to return to normal level, as opposed to nineteen days in the control. In a third experiment smaller doses of serum failed materially to affect the blood picture.

A similar experiment with hæmolytic immune serum is presented in Table XLV.

In another experiment a weaker serum failed to cause anæmia in a splenectomized dog, as well as in one with occluded splenic vein transplant; however, an animal with ligated splenic vein developed about the same amount of anæmia as the control, but took a much longer time to recover.

BEHAVIOR OF LEUCOCYTES.—Both toluylenediamine and haemolytic immune serum cause a marked leucocytosis, which reaches its height in one to three days and lasts two to four weeks. The first and greatest rise (actual and relative) is in the polymorphonuclear and transitional cells; this is later followed by a less marked and more persistent rise of small lymphocytes and eosinophiles. There was no essential difference in the reaction of the test dogs and their controls, except that the latter were affected by doses too small to influence the former. These changes resemble those previously described as following the various operative procedures on the spleen, and probably indicate a general rather than any specific interference with the leucocytic elements of the blood.

From these observations the following tentative conclusions may be drawn:

1. Dogs whose splenic vein or portal vein (Eck fistula) has been transplanted into the inferior vena cava, or whose splenic veins have been ligated, develop a lessened tendency to jaundice similar to that exhibited by splenectomized animals.
2. Although the previously existing anæmia and the concomitant increased resistance of the red cells of these animals are undoubtedly factors in the greater resistance to haemolytic agents, the lessened tendency to jaundice is, in part at least, due to a mechanical factor dependent on the change in the blood supply to the liver.

3. The additional anaemia caused in the test animals by haemolytic agents is usually less than in the controls, although the total fall from the original normal may be, and usually is, greater than in the control. This applies to the splenectomized as well as the other test animals of these experiments.

4. Although the destruction of blood in these animals is less than in the controls, the repair of the same takes considerably longer than in the controls. This confirms similar results previously obtained in splenectomized animals.

5. The white cells exhibit much the same changes as follow the administration of haemolytic agents to splenectomized or normal animals. As these changes are not unlike those following uncomplicated splenectomy or the operations here discussed, they cannot be considered as characteristic of any of the above procedures.

CHAPTER VI

THE CHANGES IN THE BONE-MARROW AFTER SPLENECTOMY

- (1) DISCUSSION OF THE LITERATURE, (2) HISTOLOGICAL STUDIES OF THE DOG'S NORMAL MARROW, (3) CHANGES AFTER SPLENECTOMY

IN many instances we have examined ³⁴⁰ the bone-marrow of splenectomized dogs with a view to determining the compensatory or other changes following the removal of the spleen. The material at our disposal consists of marrows representing periods varying from a few days to twenty-two months after splenectomy.

In the literature of the subject the reference to changes in the bone-marrow following splenectomy are for the most part casual and presented but incidentally in connection with the associated changes in the lymph- and hæmolympgh-glands. In Warthin's ⁴⁶¹ collection of the literature up to 1903 the following references occur:

Tizzoni and Fileti ⁴³⁹ (1880) and Tizzoni ⁴³⁸ (1882) observed in splenectomized dogs a transformation of the fatty marrow of long bones into red marrow.

Mosler ³⁰³ (1882), working likewise with dogs, concluded that, following splenectomy, there may be compensatory action on the part of both lymph-glands and bone-marrow, the latter appearing to play an important rôle. In one animal the bone-marrow, ten months after splenectomy, resembled that of leukæmia. This change, however, was not constant.

Laudenbach ²⁴⁰ (1893) observed in one dog, ten to

twelve years of age, with severe anaemia, signs of increased blood formation in the marrow 145 days after splenectomy.

Ceresole⁶⁹ (1895), on the other hand, found in splenectomized rabbits no clearly defined new formation of the marrow.

Warthin⁴⁶¹ ((1903) states that after splenectomy in the sheep and goat slight lymphoid changes in the fatty marrow occur, but gives no detailed histological description. Of these changes he says: "The beginning lymphoid changes in the fatty bone-marrow in the second and fifth months after splenectomy is to be regarded as compensatory only for the increased destruction of red blood-cells and not for any abrogated splenic function of red-cell formation."

Other references may be found to changes in the bone-marrow in the presence of diseases of the spleen in man and in experimental anaemias of animals with or without splenectomy, but few findings after simple removal of the normal spleen are available. Among the latter are Pugliese's³⁶³ observation that after total splenectomy the bone-marrow of the hedgehog becomes filled with giant-cells. This change Foa¹¹⁹ has not found to be characteristic of the rabbit. Vulpius,⁴⁶⁰ who, in 1894, reviewed thoroughly the subject of the surgery and physiology of the spleen, and adds some experimental observations, supports the theory of increased activity of the bone-marrow after splenectomy. Winogradow⁴⁷⁵ found red marrow in the long bones of a dog 132 days after splenectomy, but yellow marrow was present in two after 517 and 760 days respectively, though one of the latter was slightly streaked with red.

Hodenpyl,¹⁸¹ in the description of a case of absence

of the spleen in man makes no mention of the bone-marrow.

Taylor¹²⁹ describes the marrow of two splenectomized dogs: that from an animal receiving albumoses by mouth and by hypodermic injection, and killed after nine months, was red; a second, not receiving albumoses, showed a yellow marrow at the end of one year.

Freiberg^{127a} states that he found red marrow in splenectomized animals, and Gibson¹⁴⁰ notes that in a dog killed five and a half months after being deprived of the spleen the marrow was apparently in the process of change from yellow to red.

In some of these accounts brief mention is made of the increase of giant-cells or of pigmented cells or of the numerical relations between the myelocytes and the white and red cells, but we have been unable to find an adequate account of the histology of the bone-marrow after splenectomy based on modern conceptions of the cytology of this tissue. Histologic descriptions exist, but they are either brief and fragmentary or are based on views current before the attainment of our present detailed knowledge of the morphology of the cells of the blood.

METHODS.—Our studies are based chiefly on the changes in the marrow of the long bones, and particularly in that of the femur. As this marrow in the adult is normally fatty, objection may be raised against its use, and to overcome this objection we attempted to study the marrow of the compact bones. The methods of decalcifying tissues have, however, in our hands failed to yield satisfactory histologic preparations. The alternative, the use of film preparations, obtained successively at intervals over long periods of observations, being impracticable, the study of cover-glass preparations was limited to a single observation

at the time of the death of the animal. At the same time, however, in many instances marrow squeezed from the ribs has been obtained in sufficient amount to section and thus to allow a comparison with changes in the fatty marrow. We have, however, depended largely upon the study of sections of the marrow of the long bones, and in particular of the femur. We are satisfied, as the result of our study of the marrow from a large number of normal dogs, that this is, after all, the most rational method of studying compensatory changes, for it is unusual, even in a definitely fatty marrow, not to find numerous centres of blood-forming cells. These may be limited to the periphery of the marrow or be scattered throughout, but, whatever their position, they afford an excellent starting-point for the study of increased cellular content, as well as of changes in the character of the cells. The fatty marrow is of especial value in the study of the late changes, for in well-fixed and well-stained marrow there can be no doubt about the change from a purely fatty marrow to a red marrow rich in cells. This is so striking as to remove all the doubt which exists when one examines the marrow of compact bone, as of the ribs or vertebræ, by either the section or cover-glass method.

We have worked exclusively with the marrow of the middle third of the femur, avoiding the marrow at either end, partly on account of its bony nature, but chiefly because of the occasional normal occurrence of more or less red marrow at the ends of the shaft. As only adult dogs have been used, we feel that the constant use of the middle portion of the marrow gives fairly comparable results. In removing the marrow half the circumference of the bone through the greater part of its length has been chipped

away, and after separating the marrow from the bone and cutting it at either end it has been easily removed as a solid cylinder by gently rolling it on to a piece of filter-paper. In carrying these tissues through the process of fixation and imbedding, the filter-paper, which is firmly adherent to the marrow through the coagulation of the attached blood, allows the necessary manipulations without injury to the marrow itself. The routine procedure has been to fix in Zenker's fluid without previous decalcification, imbed in paraffine, and stain with eosin and polychrome methylene blue. Other stains have, however, been used whenever necessary to bring out certain details.

RESULTS.—It may be stated at the outset that we have found no evidence of an early change in the bone-marrow. Splenectomy does not cause, as do successive hemorrhages and haemolytic poisons, a rapid change of fatty marrow to red marrow. This latter change we have produced readily and rapidly in non-splenectomized control animals by the use of specific haemolytic serum and by causing hemorrhage, but we have never seen a frank change from yellow to completely red marrow in the ordinary course of events in the splenectomized animal until many months, usually six or more, had elapsed, and this despite the fact that many of the animals have had, as has been shown in our earlier work, a moderately severe anæmia. This anæmia has frequently been of as severe degree as that caused by several successive hemorrhages in the normal dog, but changes in the marrow analogous to those caused by hemorrhage have not been evident in the earlier periods following splenectomy.

In this connection it may be recalled that the anæmia of splenectomy in the dog follows a gradual downward

course for three to six weeks, the decrease in haemoglobin being relatively more marked than the decrease in red cells, and that an equally gradual repair causes the red-cell count and haemoglobin content to approach normal after three to four months or more. At the same time there is a transient initial leucocytosis, due chiefly to polymorphonuclear leucocytes, and sometimes lymphocytosis with a late eosinophilia. Not infrequently the eosinophiles disappear from the circulating blood from the third week until the end of the third month.

We have, therefore, in the course of our studies attempted to determine whether the hyperplasia in the bone-marrow after splenectomy is compensatory in the sense of (1) an overactivity in red-cell formation chiefly, (2) overactivity in the formation of the white cells chiefly, (3) an overactive, orderly reproduction of a new marrow, with involvement of all cells arising within it.

THE NORMAL MARROW OF THE FEMUR OF THE DOG

In our study of the marrow of both normal and splenectomized animals we have used as a basis for orientation Bunting's⁶³ conception of erythrogenetic and leucogenetic centres, Muir's³⁰⁶ descriptions of erythroblastic and leuco-blastic reactions, and have received also much aid from Dickson's⁹³ study of the cytology of marrow. The arrangement described by Bunting is by no means a constant and definite one, but in the masses of marrow cells may be seen groups composed mainly of myeloblasts and surrounded at times by a nearer zone of myelocytes and an outer zone of leucocytes; in other groups with the same centre the outer zone may be made up of nucleated red cells, with a still more distant zone of normocytes. We

are not convinced that centres for the production exclusively of red cells or of white cells exist, for frequently an intermingling of the two types is seen in one centre, but this conception of definite centres is of great assistance in the interpretation of marrow changes.

The study of the marrow of the femur from many normal dogs has led to our recognition of four definite groups of cells:

1. Groups of undifferentiated cells and myelocytes. These lie between fat cells and seem to be in no way connected with blood-channels. In all these centres the cells of the connective-tissue reticulum are in evidence.

2. Groups of the character described above, but with a peripheral accumulation of cells in which those of the leucocytic series predominate.

3. Groups as in (1), but with a mantle of cells in which those of the erythrocytic series are most in evidence; and

4. Groups as in (1), but with an indiscriminate mingling of cells of red and white series.

These groups cannot always be differentiated, for not infrequently an indiscriminate mingling of cells obscures the recognition of centres. Moreover, at times may be seen groups composed purely of white cells or of red cells without myeloblastic centres. We have, however, found that search for the groupings described greatly facilitates the study of complex marrow pictures and leads readily to a decision as to whether leucoblastic or erythroblastic activity predominates.

In one respect the study of normal marrow has not helped us greatly. Megakaryocytes and polykaryocytes are so infrequent in the normal fatty marrow that we have no basis, in regard to them, for a comparison with hyper-

plastic marrow. The same holds true for the large endothelial cells which are phagocytic for red cells and are found so frequently in hyperplastic marrow to contain remnants of red cells and particles of pigment.

THE MARROW OF SPLENECTOMIZED ANIMALS

In Table XLVI the general results of our observations are presented. The terms "yellow" and "red" refer to the gross appearance, not of the surface of the marrow,

TABLE XLVI
HYPERPLASIA OF THE MARROW OF THE FEMUR AFTER SPLENECTOMY

Dog No.	Period after splenectomy	Gross appearance	Microscopic change	Blood picture shortly before autopsy	
				Hæmoglobin	R. B. C.
50	24 days	Yellow	Slight	88	4,510,000
23	39 days	Yellow	None	88	6,050,000
21	40 days	Yellow	Slight	65	2,970,000
86	42 days	Yellow	Slight	96	5,820,000
79	60 days	Yellow	Slight	92	5,680,000
82	63 days	Slight streaking	Slight	76	4,530,000
17	84 days	Yellow	Slight	56	3,850,000
10	6 months	Red	Complete	78	4,410,000
39	7 months	Red	Complete	68	4,040,000
32	8 months	Yellow	Slight	97	6,120,000
44	9½ months	Yellow	None	81	4,970,000
41	10 months	Yellow	None	92	4,920,000
24	1 year	Red	Complete	110	6,048,000
59	1½ years	Red	Complete	101	5,100,000
57	1½ years	Streaked	Partial	110	5,206,000
33	1¾ years	Red	Almost complete	70	4,480,000
51	1½ years	Yellow	Slight	110	6,200,000

but of the cross-section. "Slight streaking" and "streaked" refer to an intermingling of yellow and red marrow. A marrow is described as "red" only when it is uniformly so. As will be seen by a comparison of gross and microscopic appearances, a marrow "yellow" to the naked eye may, microscopically, show evidence of beginning hyperplasia. The early changes are indicated by the

word " slight." The word " complete " indicates that only an occasional fat cell is seen microscopically. " Almost complete " means that fat cells occupy less than one-tenth of the marrow space in the surface area of sections studied. Several purely fatty marrows representing periods between five and twenty-four days after splenectomy are not included in the table.

The bone-marrows representing the earlier periods of splenectomy, in that they show practically no changes, may be dismissed briefly. This is true of a series from animals killed at various intervals from five days to three months. Some of these marrows cannot be distinguished from those of the normal dog. In others, slight replacement of fatty tissue is seen. Thus one representing the twenty-fourth day shows here and there between the fat cells single rows of blood-forming cells, with now and then clumps of ten to thirty or more. These areas are neither purely erythrogenetic nor purely leucogenetic, though in some of the groups with an older type of cells there is a predominance of polynucleated cells. The endothelial cells of the reticulum not infrequently contain large masses of old blood-pigment.

Another, representing the fortieth day, presents practically the same appearance, with a tendency, however, to greater erythrogenesis. On the other hand, a thirty-nine day dog shows a simple fatty marrow with no evidence of active blood formation. Three other marrows of this period, however, show already the early stages of hyperplasia; both types of cell groups can occasionally be isolated, but usually the groups are mixed. Greater numbers of eosinophile cells, both myelocytic and polymorphonuclear, are present than have been evident in earlier periods.

A number of cells throughout the section correspond to Longcope's²⁵⁴ small lymphocytes, and a smaller number to Longcope's large lymphocytes. The small lymphocytes are not, however, in pure groups. Polymorphonuclear leucocytes are abundant, and the picture, as a whole, is one of leucogenesis rather than of erythropoiesis. Very few giant-cells are seen, and only occasional phagocytes.

In another marrow of the sixty-third day a moderate peripheral hyperplasia of mixed type is present. Marked congestion is evident between the fat cells, and hyperplasia is seen, in places, near the periphery; in some instances the erythrocytes appear to be outside the vessel, forming distinct areas of hemorrhage. A few phagocytes are present, but giant-cells are rare. Polymorphonuclears are frequent and of mature development. At the periphery erythropoiesis seems to predominate over leucogenesis. Eosinophiles and lymphoid cells are not conspicuous.

A marrow of the sixtieth day shows less hyperplasia, but leucocytic reaction is more evident, though erythropoiesis is active. Scattered throughout the section are many small lymphocytes, but nowhere are these seen in solid clumps. Numerous deposits of pigment are seen.

Again, on the eighty-fourth day, an essentially fatty marrow shows a narrow cellular strip at the periphery in which erythropoiesis is quite active. Here and there leucogenesis predominates, but in the main the process is erythropoietic. A few nucleated red cells of the megaloblastic type are found, but the more mature normoblasts are more abundant. In some centres radiating lines of four or five normoblasts are seen. Few giant-cells are present.

The marrows of the fourth and fifth months after

splenectomy are not represented in this study. Well-marked hyperplasia is, however, present in bone-marrow representing periods of six, seven, twelve, seventeen, eighteen, and twenty months after splenectomy. On the other hand, two marrows representing respectively nine and one-half and ten months show no departure from the normal fatty marrow, and in a third (eighth month) only slight hyperplasia is evident. In the latter are areas composed almost entirely of cells of the myelocyte or pre-myelocyte type, with some evidence of the formation of both red cells and polymorphonuclear leucocytes. The picture suggests a proliferation of the primitive cells of the marrow, without, however, a very active further differentiation. In a fourth animal of the late period (twenty-second month) only slight hyperplasia was present. With evidence of well-marked hyperplasia in other animals at six and seven months after splenectomy and after a year and a half, it is impossible to explain its failure in these four animals representing the eighth, ninth, tenth, and twenty-second months respectively.

The best opportunity of studying the late changes is presented by material from six animals, representing the period from six to twenty months, in all of which the fatty marrow of the femur was transformed entirely or in large part into red marrow. The histological picture of each of these will be given in detail.

Dog 10.—Splenectomized May 20, 1913. Before operation the red cells numbered 6,910,000 and the haemoglobin was 105 per cent. The severest anaemia was reached July 21: red cells 4,240,000, haemoglobin 62. On September 11th the figures were 5,220,000 and 92. Later the animal became pregnant and anaemia recurred, the picture on No-

vember 18 being red cells 4,410,000, haemoglobin 78 per cent. On November 24 the animal was chloroformed. At autopsy the medulla of both femurs presented a deep-red marrow.

Histologically is seen a uniformly cellular tissue, with only occasionally a fat space here and there at the periphery. For the most part this marrow is as definitely cellular as is, for example, a lymph-node or the spleen, and indeed, it has much of the appearance of the pulp of the latter organ in the new-born puppy. In this cellular mass, which at first appears to present a hopeless confusion of cells, it is not difficult to resolve the cells into more or less distinct proliferating centres. The arrangement is by no means a definite one, but in the patchwork of cells one sees groups which correspond to Bunting's description. In speaking of these centres we will refer to them as erythropoietic or leucopoietic, according to whether red cells or polymorphonuclear leucocytes predominate in the mass of cells surrounding the centre in question. We have made no attempt to distinguish in these centres, which may include from six to thirty cells, between the finely granular neutrophilic myelocyte and the non-granular basophilic cell from which it is supposed to arise. In these centres mitotic figures may occasionally be seen, but only after prolonged search. It is also in these centres that old blood-pigment, which is quite abundant in this marrow, is deposited; its deposition in the loose vascular tissue elsewhere has not been observed. The erythropoietic centres appear to be more active than the leucopoietic. This impression is based on the fact that about a mass of myeloblasts, composed of twelve to fifteen cells, may be seen twenty-five to thirty nucleated red cells and a small

number of normocytes, while about the leucogenetic centres comparatively few leucocytes are seen. The red cells in question vary in size and show intermediate stages from the megaloblast to the normocyte. It is not to be supposed that about erythrogenetic centres no leucocytes occur; a few are always present. For example, among the twenty-five to thirty cells mentioned above, eight polymorphonuclear leucocytes could be clearly distinguished. Sometimes on one side of a centre nucleated erythrocytes may be grouped, and on the other leucocytes, with little intermingling. This suggests simultaneous formation of the two cells in one cell centre. When this occurs the number of red cells is always greater than the number of leucocytes, in proportion of about 4 to 1. All through the section are lymphoid cells, usually single and of the small variety. Giant-cells are frequent, and a few show inclusions of polymorphonuclear leucocytes. Cells containing such inclusions have a broad, homogeneous gray staining protoplasm suggesting necrosis. There is considerable pigment, but not many phagocytic endothelial cells are seen. Normoblasts are seen free in the capillaries. Smears from the marrow of the ribs show active erythropoiesis and, on the whole, much the same cellular picture as the marrow just described. In the rib-marrow a considerable number of eosinophiles, chiefly polymorphonuclears, are also seen. In connection with the activity in the formation of red cells shown by the marrow, it is significant that the blood count six days before death was 4,100,000 and the haemoglobin 78 per cent. (on September 11, four months after splenectomy, the figures were 5,240,000 and 92). In other words, despite the hyperplasia of the bone-marrow, the animal exhibited a late anaemia, two and a half

months after recovery from the initial anaemia, following splenectomy. This may have been due to the drain occasioned by the intervening pregnancy—an unfortunate complication from the point of view of the study of the blood. The fact remains, however, that the marrow is actively forming normal red cells. The anaemia was, therefore, not due to abnormally low erythropoiesis in the marrow.

Dog 39.—On April 7, 1913, before splenectomy, the red cells numbered 6,528,000, and haemoglobin was 110 per cent. The lowest point of anaemia was reached on June 3, the red cells numbering at that time 3,650,000; the haemoglobin was 62 per cent. By July 7 the blood picture had improved (red cells 5,080,000, haemoglobin 88 per cent.), but on September 11 a late recrudescence of the anaemia gave red cells 4,040,000 and haemoglobin 68 per cent. The animal was killed on November 15. The bone-marrow of the femur was of a definite red color. The anaemia did not affect the general nutrition of the animal, for on April 7 the weight was 12,800 gms.; on November 15 it was 13,950, and the adipose tissue was abundant.

This animal, representing practically the same period after splenectomy and the same changes in the blood as Dog 10, gives very much the same picture in the marrow. Of minor importance is the fact that the marrow is not so cellular, the proportion of cells to fat being in the ratio of about 3 to 2; also, the myelocytic centres are not so pronounced, but in other respects the marrow is the same. Many giant-cells are present, but lymphoid cells are rare. The formation of red cells and leucocytes is perhaps not so rapid (that is, the numbers about any one centre are not so great), but, on the other hand, the erythropoietic

activity of the marrow is sufficient to exclude the possibility of an inactive bone-marrow being responsible for the late recrudescence of anaemia.

Dog 24.—This animal was splenectomized on February 10, 1912, and was used for the injection of haemolytic immune serum on March 20 and again on April 7. On June 28 it had recovered from the anaemia then produced (red cells 5,650,000, haemoglobin 89 per cent.), and on July 15 it was treated with sodium oleate. On September 26 the red-cell count was 5,780,000 and haemoglobin 90 per cent. On February 19, 1913, the blood picture had improved (red cells 6,048,000, haemoglobin 110 per cent.), and at this time haemolytic serum was again injected. The animal was chloroformed on March 4, 1913. The lapse of time since splenectomy was, therefore, thirteen months. At autopsy a red marrow was found.

Owing to the use of various haemolytic poisons, the bone-marrow of this animal may have been influenced by other factors than the absence of the spleen. The histological picture, however, is so in accord with the marrow of simple splenectomy that, with this explanation, it is included in the series.

HISTOLOGY.—The marrow is a uniformly solid marrow, with no fat spaces visible in any of the sections examined. It does not, however, appear to be as cellular as the marrow of Dogs 10 and 39. This difference is caused by a greater congestion and distention of the blood-vessels, a slight increase in the reticulum, and a lessened tendency of the myelocytic tissue to be grouped in large centres. Erythroblastic centres are very prominent and very active; leucogenetic centres, on the other hand, are made out with difficulty. Lymphoid elements are rare. Many cells of

the myelocytic type are seen with coarse basic granules and short threads in the nucleus and with little or no protoplasm. In close relation to these are sometimes seen degenerated mitotic cells, but whether all the chromatin masses can be so interpreted is not clear. These degenerative changes are doubtless the result of the last injection of haemolytic serum.

Dog 59.—On July 24, 1912, this animal was splenectomized and used for the study of the progressive anæmia following this procedure. On December 7, 1912, the highest point (red cells 5,250,000, haemoglobin 105 per cent.) of recovery was reached. Continued observation showed a slight decline to 5,200,000 red cells and 86 per cent. of haemoglobin on May 21, 1913, on which date the animal was used in an experiment with sodium oleate. From the moderate anæmia caused at this time the animal recovered, the blood examination on June 9 showing 5,050,000 red cells and 86 per cent. haemoglobin, the condition slightly improving as to haemoglobin content until November 18, 1913, when red cells were 5,100,000 and haemoglobin 101 per cent. The animal was chloroformed on November 24. At autopsy the bone-marrow of the femur was soft, succulent, and dark red in color. In connection with the general condition of this animal it is of interest to note that in the last seven months its weight increased from 10,450 grammes to 12,580 grammes, and that adipose tissue was very abundant. The administration of sodium oleate introduces a possible disturbing factor, but as this was given six months before death, and as the anæmia which it caused was quickly repaired, it is not considered, in view of our other observations, to have had an important influence on the bone-marrow.

HISTOLOGY.—The marrow shows some fat cells, the proportion of marrow cells to fat being about 10 to 1. Nothing different from the last four marrows is presented. Leucogenesis and erythrogenesis proceed at about equal rate, the latter being a little more active. Mitotic figures are seen not infrequently, but the type of cell in which they occur is not always evident. Myeloblasts seem to be more abundant than usual. Giant-cells are fairly abundant, but lymphoid cells are rare.

Dog 57.—On June 23, 1912, the blood of this dog contained 5,350,000 red cells per cubic millimetre and 98 per cent. hæmoglobin. On July 2 the spleen was removed. The resulting anæmia reached its lowest point (red cells 2,970,000, hæmoglobin 50 per cent.), on August 5. On October 24, when the blood count showed 5,240,000 red cells and 90 per cent. hæmoglobin, the animal received sodium oleate intravenously; a slight anæmia (fall in hæmoglobin to 62 per cent., but no marked change in red cells) resulted. In January, 1913, the red cells were 5,206,000, hæmoglobin 110 per cent., and with slight variations this higher level was maintained, accompanied by an increase in body weight, until December 12, 1913, when the animal was chloroformed. At autopsy the animal was found to have a large amount of adipose tissue; the bone-marrow of the femur was definitely reddish in color, with faint yellowish streaks. As the sodium oleate given four months after splenectomy and fourteen months before death produced only a slight transient change, we consider that the bone-marrow represents the effect of splenectomy only.

HISTOLOGICAL EXAMINATION.—The relation of the fat to cells is about 1 to 1; otherwise nothing new is seen. The marrow is very active, leucogenesis and erythrogenesis

being equally prominent. Phagocytic cells and masses of old blood-pigment are quite numerous, as are also giant-cells. More abundant than in other marrows are eosinophiles of the myelocytic type. Lymphoid cells are not conspicuous.

Dog 33.—This animal was splenectomized on May 14, 1912. The blood examination on the previous day showed 4,950,000 red cells and 85 per cent. haemoglobin. The anaemia following splenectomy reached its lowest point on June 28 (red cells 3,550,000, haemoglobin 52 per cent.). On September 20 the red cells had risen to 5,490,000 and haemoglobin to 95 per cent. In November, 1913, the animal passed successfully through pregnancy. In January, 1914, as the animal had developed mange, it was chloroformed. The blood examination on the preceding day was red cells 4,480,000, haemoglobin 70 per cent. At autopsy the bone-marrow of the femur was deep red in color. (It should be stated that one and two months before splenectomy the animal had received injections of haemolytic serum. From our studies of the effect of haemolytic serum in the normal dog, we do not believe that these injections, several weeks before splenectomy and nearly two years before death, are in any way responsible for the hyperplasia of the marrow.)

HISTOLOGICAL EXAMINATION.—This marrow differs in no way from the marrows of Dogs 57 and 59 described above.

Dog 51.—The spleen was removed on May 31, 1912, and on June 26 of the same year haemolytic serum was administered. From the anaemia thus produced the animal made a slow recovery, but after 200 days the blood examination showed 6,200,000 red cells and 110 per cent. haemo-

globin, as compared with 6,210,000 red cells and 100 per cent. haemoglobin before splenectomy. On March 26, 1914, when the animal was chloroformed, its weight was 9750 grammes, as compared with 8270 grammes at the time of splenectomy and 8120 grammes when haemolytic serum was administered. The notes made at the autopsy refer to the large amount of adipose tissue, the normal appearance of the lymph-nodes, the absence of supernumerary spleens, and the presence in the long bones of a distinctly yellow, fatty marrow. Histological examination of the marrow shows a very slight hyperplasia, with large numbers of leucocytes and deposits of blood-pigment.

DISCUSSION.—It will be remembered that in discussing the anaemia which follows splenectomy it was pointed out that nucleated and other abnormal forms of red cells are rarely found in the circulating blood, and that when they do occur they do not persist for any length of time. Apparently their appearance corresponds to the beginning repair and not to the period of hyperplasia of the marrow which we have described. It is difficult, therefore, to bring the changes in the bone-marrow into relation with the changes in the peripheral blood. If the hyperplasia of the bone-marrow is compensatory to increased blood destruction or decreased blood formation, one would expect definite hyperplasia to be present in the earlier period, during the first three months after splenectomy, at a time when the anaemia is evident and repair is taking place, and not after six months to a year or a year and a half, when the blood picture is normal. It is true that in two of the animals (Dogs 10 and 39) a late recrudescence of anaemia occurred and the marrows of these animals were obtained during this period, but this was not the case in most of the animals

of the series and is not characteristic of the late periods after splenectomy. It is therefore impossible, on account of the late development of hyperplasia in the marrow, to explain its occurrence as compensatory to the anaemia following splenectomy.

Likewise we cannot accept Warthin's⁴⁶¹ theory based upon his study of sheep and goats. In these animals Warthin found hyperplasia of the marrow to occur several months after splenectomy and to be associated with evidence of increased destruction of red blood-cells in the lymph- and haemolymph-nodes. This destruction, greater than that in the primitive spleen, is responsible, he believes, for the anaemia following splenectomy, and this is in turn compensated by increased activity in the bone-marrow. We have found little to support this theory in our studies of the dog. The lymph-nodes, as well as the endothelial cells of the liver, as we have shown elsewhere,³³³ are indeed more active in the phagocytosis and destruction of red cells after splenectomy than in the normal animal, and this is very evident when large numbers of red cells are injured, as by the administration of a haemolytic poison; but in the ordinary course of events, after splenectomy, the lymph-nodes present no evidence of excessive blood destruction. An occasional cell containing one or two red cells may be seen, and small amounts of old blood-pigment are occasionally demonstrable, but of excessive haemolysis there is no evidence. Likewise, microchemical tests for iron in the lymph-nodes and liver show that little difference exists in this respect between the normal and splenectomized animals. For this reason, and because the anaemia is not persistent and progressive, we cannot support the theory that the hyperplasia of the marrow is compen-

satory to abnormal blood destruction in the lymph-nodes.

Another possible explanation is that the bone-marrow, in the absence of the spleen, is concerned in the storing and utilization of iron. There is no doubt that, in the normal animal, iron set free in the dissolution of red cells is stored in the spleen. After splenectomy a readjustment in the storage of iron takes place, and there is some evidence that for a short time after the removal of the spleen iron may be lost from the body. Our investigations²² show, however, that this disturbance of iron utilization is transient, and that after a few weeks the elimination of iron in the splenectomized animal differs in no way from the process in the normal animal. This suggests naturally that the storage of iron in the absence of the spleen is taken over by other tissues. As microchemical tests for iron showed no definite increase of iron in the lymph-nodes and liver, it seemed probable that the bone-marrow might be the chief depot of iron storage. Such a view was supported by the fact that all hyperplastic bone-marrows contain large amounts of altered blood-pigment, sometimes free, but occurring, for the most part, in large phagocytic cells. The activity of these phagocytic cells in transforming the iron of old blood-pigment in order that it may be utilized for red cells might, it was plausible to suppose, stimulate the other functions of the bone-marrow (that is, the erythrogenetic and leucogenetic functions) and cause eventually a replacement of the fatty marrow by a very cellular red marrow.

In order to prove this hypothesis it was necessary to obtain some idea of the iron content of these marrows. Direct chemical analysis was out of the question on account of the small amount of material available and the varia-

tions in blood and bone content of different marrows. We therefore made a comparative study based on the use of the microchemical reaction for iron. This demonstrated at once that all red marrows in our series have a large content of iron, and that fatty marrows contain very little or no iron. On the other hand, when the marrows of non-splenectomized dogs rendered hyperplastic by anaemia or infection were examined it was found that these also had a large iron content. Thus in a group of seventeen non-splenectomized dogs iron was present in the marrow in large amounts in four, in moderate amounts in two, in small amounts in four, and in seven none was found. On the other hand, in twenty-seven splenectomized dogs, iron was present in large amounts in ten, in moderate amounts in three, small amounts in four, and absent in ten.

In both groups the amount of iron was in direct proportion to the degree of hyperplasia. These observations point, therefore, to the conclusion that a red marrow is always rich in iron, but it is impossible to say whether the cellular hyperplasia or the iron deposition is primary. Under the circumstances, it is also impossible to conclude that the late hyperplasia of marrow following splenectomy is an attempt to conserve iron. Moreover, the irregularity of our results, as shown by the failure of hyperplasia in four animals, representing respectively the eighth, ninth, tenth, and twenty-second months after splenectomy, prevents, in the present state of our knowledge, an adequate explanation of the cause of the transformation from yellow to red marrow. However, the tardiness with which hyperplasia of the bone-marrow appears after splenectomy, despite the presence of an anaemia of considerable grade, may well bear some causative relation to the slow degree of

blood regeneration that follows the administration of haemolytic agents to such animals.

The divergent results in this study are characteristic of all phases of experimental work on the spleen, and doubtless are to be explained by the fact that removing the spleen takes away only one organ of a system composed of liver, spleen, lymph-nodes, and bone-marrow, and that the interrelations which exist in this system may, or may not, under varying circumstances, bring into play compensations of the greatest importance in determining the degree of blood destruction or regeneration and therefore the degree of change in the bone-marrow.

A search of the literature of splenectomy in man, although it reveals evidence of the occurrence of red marrow in various forms of splenic anaemia, offers little of importance concerning the changes which occur in the bone-marrow after removal of the normal spleen. Several references are made to the occurrence of pain in the long bones after splenectomy, and by some this has been assumed to be evidence of hyperplasia within the rigid bony canal. The only note of the direct examination of the bone-marrow after splenectomy is that of Riegner,³⁷⁵ who found active proliferation of the marrow of the femur in a man whose leg was amputated for gangrene four weeks after splenectomy for trauma. It is therefore impossible, on account of this paucity of data concerning the changes in man, to bring them into relation with our experimental results.

CONCLUSIONS.—Splenectomy in the dog causes, as a rule, a transformation of the fatty marrow of the long bones into a richly cellular red marrow.

During the early periods, one to three months, the change in the marrow is slight and either focal or pe-

ipheral; after six to twenty months the replacement of fat by marrow cells is complete or nearly so. Exceptions were, however, seen in four animals representing the eighth, ninth, tenth, and twenty-second months respectively.

The evidence at hand does not support the theory that this hyperplasia is compensatory either to the anaemia caused by splenectomy or to an increased haemolysis in the lymph-nodes. It is possible that it may be a concomitant of the activity of the bone-marrow in taking over, in the absence of the spleen, the function of storing and elaborating the iron of old blood-pigment for future utilization by new red cells, but our studies do not fully support this view.

CHAPTER VII

THE CHANGES IN THE LIVER AND LYMPH-NODES AFTER SPLENECTOMY

DISCUSSION OF THE LITERATURE. CHANGES IN LYMPH-NODES. PHAGOCYTOSIS OF RED CELLS BY ENDOTHELIAL CELLS OF LYMPH-NODES AND LIVER. MICROCHEMICAL TEST FOR IRON IN LYMPH-NODES AND LIVER.

MUCH of the early literature concerning compensatory changes in the lymph-nodes is bound up with the question of the regeneration of the spleen after partial or complete extirpation and more recently with problems concerning the hæmolympmph-nodes. As this literature has been collected very completely by Warthin,⁴⁶¹ we will give only a general summary of it in the light of his investigation. The earliest detailed investigations are those of Tizzoni and Fileti,⁴³⁹ who observed in the splenectomized dog an increase in size of the retroperitoneal and thoracic lymph-nodes, which were distinctly red in color. In two dogs, splenectomized for fifty-four days and three and a half months respectively, they found a new formation of spleen-like nodules in the omentum. These they believed to be formed directly from adipose tissue by an absorption of fat and a transformation of the fat cells into reticulum. Leucocytic infiltration followed, as also proliferation of endothelium, which produced eventually a pulp-like tissue, in the meshes of which were red blood-cells. Around the whole a connective-tissue capsule was formed. Later, in

1882, Tizzoni⁴³⁸ found somewhat similar bodies in the gastrosplenic ligament, in connection with indurative splenitis in the dog, and after splenectomy numerous newly-formed nodules throughout the subperitoneal fat, over the diaphragm, and in the pelvic, sterno-abdominal and subcutaneous fat tissue. Foa,¹¹⁹ in 1883, denied the new formation of such nodes, and explained them as preexisting nodes, changed in color by hemorrhage or other pathological conditions. In the meantime, however, Winogradow⁴⁷⁵ had described in dogs killed 132, 517, and 760 days after splenectomy changes in existing lymph-nodes similar to those observed by Tizzoni. On account of the presence of red cells and pigmented cells in the sinuses of these nodes, he believed these structures to have a share in blood destruction, and that possibly the anaemia occurring after splenectomy could in this way be explained.

Zezas⁴⁸⁵ found that after splenectomy the mesenteric and bronchial lymph-node of the rabbit became swollen, dark red in color, and firmer in consistency, and quotes Hegar and Simon as finding similar changes in the mesenteric lymph-nodes of the cat. Tizzoni⁴³⁸ and Ceresole⁶⁹ could not confirm these changes in the rabbit. Mosler³⁰³ found in a dog, splenectomized ten months, numerous spleen-like nodules of the size of a pea, scattered throughout the greater and lesser omentum. Microscopically, these were similar in structure to those found by Tizzoni and Winogradow, but Mosler regarded them as neoplasms—hemorrhagic telangiectatic lymphoma—and not as newly-formed spleen or lymph-nodes. The lymph-nodes generally were not hyperplastic, and the bodies described above were not constantly present; in one dog, for example, killed after eleven months, no changes in the lym-

phoid tissue were found. Mosler concludes, nevertheless, that after splenectomy compensatory changes may occur in lymph-glands and bone-marrow.

Gibson¹⁴⁰ found in splenectomized dogs enlarged mesenteric lymph-nodes containing both normoblasts and normocytes in their sinuses. Of other investigators, Eternod¹⁰⁷, found in a fox, 161 days after splenectomy, a splenic nodule in the omentum and newly-formed lymph-nodes in the mesentery, the other lymph-nodes being enlarged and of a brownish color; Vulpius⁴⁶⁰ found no enlargement of lymph-nodes in dogs dying after a few days, or killed five months after splenectomy; Laudenbach²⁴⁰ found that hyperplasia of the lymph-nodes was not constant, and that evidence of increased blood formation was present in the bone-marrow only.

An examination of Warthin's summary of the literature concerning the lymph-nodes of man after splenectomy shows that no constant changes have been observed. Temporary local enlargement of lymphoid tissue has been observed and occasionally general enlargement; on the other hand, in many cases no changes whatever have been seen.

In the congenital absence of the spleen, as in the case reported by Hodenpyl,¹⁸¹ general enlargement of all the lymph-nodes of the body is found, as also usually a new formation of lymphoid tissue in the adrenals and liver.

Warthin's studies⁴⁶¹ were upon sheep and goats which normally have numerous haemolymph-nodes in the pre-vertebral fat. One and two weeks after splenectomy, lymph- and haemolymph-glands presented evidence of an increased number of pigment-bearing phagocytes and eosinophiles and a proliferation of lymphoid tissue gen-

erally. At the end of two months these changes were more marked and, in addition, new formation of hæmolymph-nodes in adipose tissue was evident. The changes were progressive, and at the end of five months are described as follows: "Great hyperplasia and new formation of lymph-nodes, new formation of hæmolymph-nodes in adipose tissue, marked haemolysis, eosinophiles in the lymphoid tissues, pigmentation of the liver, and slight lymphoid changes in the fatty marrow." Leucocytosis was most marked at the end of two months.

From this summary it is evident that two types of changes have been found: (1) A peculiar new formation of lymph-nodes (Tizzoni, Winogradow and Mosler), probably identical with the hæmolymph-node described by Warthin, and (2) an inconstant hyperplasia of preexisting lymph-nodes with reddish or reddish-brown discolouration.

As to the formation or destruction of red cells by the lymph-nodes after splenectomy, divergent opinions are held. Gibson¹⁴⁰ and Laudenbach,²⁴⁰ among others, support the theory of red-cell formation; Warthin saw no evidence of this. On the other hand, the latter states that his findings indicate that the splenic functions of haemolysis and leucocyte formation are, in the absence of the spleen, taken over by the lymph- and hæmolymph-nodes. So far as the function of haemolysis is concerned, this view is supported by Morandi and Sisto,²⁹⁸ who found in the hæmolymph-nodes of dogs evidence of increased haemolysis after splenectomy. According to Warthin, "the haemolytic function of the hæmolymph-nodes and hyperplastic lymph-glands exceeds that of the primitive spleen, causing an excessive destruction of red cells. The resulting anaemia is later compensated for by an increased activity on the

part of the bone-marrow. It would appear, therefore, that the removal of the spleen leads to an increased production or retention of some haemolytic agent usually disposed of by the spleen. The effect of this haemolytic agent is either to stimulate the phagocytes in the haemolymph-nodes to increased activity, or to change the red cells so that they are more easily destroyed by these phagocytes."

This view is not shared by Banti,²⁹ who, although he considers the lymph-nodes, liver, and bone-marrow to be secondary organs of haemolysis, denies that they may compensate for the haemolytic activity of the spleen. Indeed, it is upon this argument that he bases the beneficial results of extirpation of the spleen in haemolytic splenomegaly.

CHANGES IN THE LYMPH-NODES.—All animals used in our studies of the effect of splenectomy have been carefully examined³³³ at autopsy in the hope of finding the haemolymph-nodes occasionally noted by other investigators. In this we have not been successful. Never upon gross examination have we found structures corresponding to Warthin's description, and the occasional doubtful haemolymph-node has always proved upon histological examination to be a haemorrhagic or otherwise pathologically altered lymph-node. Upon this point we have felt relieved since Dr. Warthin assured us that the dog is a very unsatisfactory animal for the study of the haemolymph-node. Still, it has been a matter of surprise to us that we have found in none of the many dogs we have examined the bodies described by Tizzoni, Winogradow, and Mosser.

The second change in the lymph-nodes—a reddish or reddish-brown discoloration—described by other observers we have frequently seen. More frequently this has been a reddening limited to the centre of the node, at other times a

diffuse reddening. In animals splenectomized ten months or more the red usually gives way to a brownish color, especially in the inguinal and axillary lymph-node.

Hyperplasia of the lymph-nodes has been common in animals killed shortly after splenectomy, but in those representing the longer periods it has been impossible to distinguish any appreciable increase in size and certainly no new formation.

On the other hand, the lymph-nodes (as also the liver) in a small group of animals presented changes which appear to be of significance in connection with the general problem of blood destruction in the absence of the spleen. These changes are (1) a proliferation of the endothelial cells, and (2) an increase in the phagocytic power of these cells for red blood-corpuscles.

It is not necessary to discuss the voluminous literature concerning the destruction of red cells. This has been well presented up to 1895 by Gabbi¹³⁵ and up to 1901 by Hunter.¹⁸⁸ The more recent literature has added little either in fact or theory that is new. Out of the mass of contradictory statements there is uniformity of opinion on only two points: (1) That large endothelial cells of the spleen (the red blood-corpuscle-carrying cells) have the power to engulf red blood-cells; and (2) that the presence (in anaemia and malaria) of blood-pigment in the cells (Kupffer's cells) of the liver capillaries indicates that these cells play some important part in the destruction of red blood-cells. On the other hand, it is not generally admitted that the endothelial cells of the lymph-nodes likewise have this power. That phagocytosis of red cells, wherever it occurs, leads ultimately to the freeing of haemoglobin, which eventually reaches the liver and is

transformed into bile-pigment, is the opinion of all who support the theory that this mechanism plays a part in the destruction of red blood-cells. There is, however, no uniformity of opinion as to whether the hæmoglobin is set free in the liver from red cells carried there by the phagocytes or whether it is set free by the phagocytes elsewhere and carried to the liver in another way.

It is only with one phase of the subject, the rôle of endothelial cells in engulfing red cells in the absence of the spleen, that we will concern ourselves here. Our hypothesis is that in the absence of the spleen the endothelial cells of the lymph-nodes and liver compensate, at times of excessive blood destruction, for the loss of similar cells of the spleen.

This possibility was first brought to our attention in the routine examination of tissues from splenectomized dogs which had received specific hæmolytic immune serum. So striking were some of the pictures that we undertook, for the sake of control, the study of the liver and lymph-nodes from a number of normal dogs, of normal dogs receiving hæmolytic serum, and of dogs which had been splenectomized for various lengths of time, but which had not received hæmolytic serum.

The literature of splenectomy offers little aid in determining the histological changes occurring in the lymph-nodes after removal of the spleen. In the literature at our disposal no definite descriptions have been found except those of Warthin, who found in sheep and goats an increase in the phagocytic power of the endothelial cells for red blood-corpuscles. Gabbi, who worked with the guinea-pig, states that a transient increase of the red blood-corpuscle-carrying cells may possibly occur in

early periods after splenectomy, but that after three to six months they are no more abundant than in the normal animal.

The lymph-nodes studied have been for the most part the mesenteric, gastrohepatic, prevertebral, and bronchial. In the normal animal these have been examined more particularly for the frequency of mitosis, for the number of endothelial cells in the sinuses, and for the presence of cells containing red blood-corpuscles. Careful study of nodes from five normal animals showed that mitotic figures are found only after prolonged search and are usually limited to the follicles. The number of endothelial cells varies, but usually is not great, and they never occur in large masses in the sinuses. These cells, however, not infrequently contain old blood-pigment, and occasionally a cell may be seen containing one or two red blood-corpuscles.

The lymph-nodes of five animals splenectomized for three, four (two), thirty-nine and eighty-four days and not subjected to the action of haemolytic serum have been studied in the same way. In two animals representing respectively four and eighty-four days the lymph-nodes differed in no way from the normal; in the other animals mitotic figures were abundant in the follicles, and the endothelial cells in the sinuses were greatly increased in number. Prolonged search, however, failed to demonstrate mitotic figures in the latter cells, and, although they occasionally contained one or two red blood-corpuscles, this power of phagocytosis did not appear to be greater than in the non-splenectomized animal. The increase in the number of endothelial cells was, however, very striking.

The lymph-nodes of five normal dogs, which had received

specific haemolytic immune serum and had died or been chloroformed, after periods varying from twenty-four hours to nine days, showed the lesions usually described as common to various cytotoxic sera²³² and especially to lymphotoxic sera.¹¹⁴ These are oedema, increase of polymorphonuclear leucocytes, focal areas of necrosis, abundant

TABLE XLVII

CHANGES IN THE LYMPH-NODES AFTER SPLENECTOMY AND THE INJECTION OF HÆMOLYTIC SERUM

Experiment No.	Period after splenectomy	Period after serum	Effect of serum	Histology
I	3 days	36 hours	Hæmoglobinuria	Extreme phagocytosis of red cells
II	300 days	36 hours	Severe jaundice	Well marked phagocytosis of red cells
III	285 days	18 hours	No record	Well marked phagocytosis of red cells
IV	225 days	48 hours	Hæmoglobinuria	Moderate phagocytosis of red cells
V	15 days	3 days	Hæmoglobinuria	Negative
VI	27 days	3 days	Hæmoglobinuria	Proliferation of endothelial cells
VII	33 days	4 days	Jaundice	Proliferation of endothelial cells
VIII	65 days	8 days	Hæmoglobinuria	Proliferation of endothelial cells
IX	6 days	9 days	Hemoglobinuria	Negative
X	3 days	9 days	No hæmoglobinuria or jaundice	Negative
XI	103 days	10 days	No hæmoglobinuria (spontaneous jaundice)	Proliferation of endothelial cells
XII	25 days	15 days	Jaundice	Proliferation of endothelial cells

mitotic figures in the follicles, and slightly greater frequency of large endothelial cells capable of phagocytosis of red cells.

Of animals that had been splenectomized and had received haemolytic serum as well, twelve were available for histological examination. Of these, three showed no change in the lymph-nodes and five showed a well-marked in-

crease in the number of endothelial cells in the sinuses, but no increase in power of phagocytosis. In the four remaining animals the sinuses contained a great number of large endothelial cells filled with red blood-corpuscles. The analysis of these findings is somewhat difficult, as three factors must be considered: (1) The length of time after splenectomy; (2) the lapse of time between administration of haemolytic serum and the death of the animal; and (3) the degree of red-cell destruction caused by the serum.

These factors are brought out in Table XLVII.

From this analysis it is seen that the proliferation of the endothelial cells did not occur in the animals (V, IX, and X) splenectomized for periods of from three to fifteen days, but was evident in five (VI, VII, VIII, XI, and XII) in which the time elapsing since splenectomy was 27 to 103 days. On the other hand, the lymph-nodes of these animals did not present evidence of increased phagocytosis of red cells. Whether this was due to the period which had elapsed (three to fifteen days) since injection of serum could not be determined, but this was probably the case.

Certainly it was not due to failure of haemolysis, for at least two of these animals (VI and VIII) presented evidence of extreme blood destruction. That the period of time elapsing may be an important factor is shown by the fact that all animals (four) in which there was evidence of extensive phagocytosis of red cells represent periods of eighteen to forty-eight hours after injection of the serum. In the absence of exact knowledge of the length of time necessary for the destruction of red cells by phagocytic endothelial cells, it is useless to surmise, but one can-

not escape the fact that in this investigation all evidence of active phagocytosis is seen in animals dying within forty-eight hours. It is possible, therefore, that the destruction of red cells by phagocytosis may be completed within forty-eight hours, and this view is supported by the frequency with which pigment is found in the lymph-nodes at later periods.

It is also evident that the time elapsing since splenectomy bears no relation to the occurrence of phagocytosis of red cells, for the most marked example of the latter was seen in a dog dying three days after splenectomy, while moderate and well-marked phagocytosis occurred likewise after seven and one-half, nine and one-half and ten months.

Recently Karsner, Amiral, and Bock²⁰⁸ have confirmed these results and have added some interesting observations concerning the time element. Their conclusions are as follows:

In cats the same phenomenon of phagocytosis of red blood-corpuscles in the liver and lymph-nodes takes place as in the dog. In the series of splenectomized animals killed three, six, twelve, twenty-four, and forty-eight hours after injection we found at three hours hyaline thrombosis, large numbers of mitotic figures, and in three lymph-nodes two phagocytes containing each one red blood-corpuscle and showing a certain amount of pigment phagocytosis; at six hours there was karyorrhexis in the central parts of the follicles, marked mitosis, phagocytosis of red blood-cells and of pigment, more marked in the central sinuses than in the peripheral sinuses. Digestive vacuoles in these phagocytes contained only a few erythrocytes. At twelve hours there was marked phagocytosis of the red blood-corpuscles in the central and peripheral sinuses, also phago-

cytosis of pigment granules. At twenty-four hours the necrosis in the follicles did not appear to be severe; the phagocytosis of the red blood-corpuses was seen principally in the peripheral sinuses. At forty-eight hours there was still hyaline thrombosis, very little evidence of necrosis, marked phagocytosis of pigment both in the central and peripheral sinuses. Practically no red blood-corpuses were within the phagocytes, although simple acidophilic granules, apparently erythrocytic fragments, were found.

The story of the phagocytosis appears to be that it begins at about three hours after injection of the immune serum, continues progressively, and reaches its height at somewhere between twelve and twenty-four hours after injection, and then the destruction of the corpuscles goes on, so that at forty-eight hours there is nothing left but pigment and corpuscular fragments. Furthermore, with the passage of time the individual phagocytes become more and more filled with erythrocytes until about twelve to twenty-four hours, at which time there is a disappearance of the erythrocytes with the substitution of the pigment granules. The origin of the phagocytes appears to be particularly the endothelial cells of the sinuses, but in many of the lymph-nodes it was found that the individual cells of some of the smaller blood-vessels were swollen and also phagocytic, and it is possible that this is an important matter in connection with the origin of the phagocytic cells and also an important way of explaining the presence of red blood-corpuses within the lymphatic sinuses. Careful examination failed to show anything in the nature of rupture of any of the blood-vessels.

Another phase of Karsner's work had to do with the question of hæmopsonins; that is, with the possibility of

the spleen having some relation to a substance which renders the red cells more susceptible, or otherwise, to phagocytosis, and the possible increase or decrease of such a substance after splenectomy. He summarizes²⁰⁸ the results of this phase of the investigation, as follows:

1. Splenectomy produces no change in hæmopsonins of the circulating blood that is clearly demonstrable by *in vitro* tests.
2. The venous blood returning from the kidney and from the spleen and the venous blood of the portal vein, of the right auricle, and of the left ventricle showed the same content of hæmopsonin.
3. Extracts of washed spleen, kidney, pancreas, and liver showed no influence over the phagocytic activity of the corpuscle, serum, and exudate mixture used in the opsonic work.
4. Extracts of the lymph-nodes of splenectomized dogs and the extract of the lymph-nodes of normal dogs have no influence over the phagocytic mixture mentioned under heading 3.

CHANGES IN THE LIVER.—Examination of the stellate endothelial cells (Kupffer's cells) of the liver²³³ has been rendered difficult on account of the intense congestion and abundant necrosis which occur in the liver after the administration of hæmolytic immune serum. For this reason we have not always been able to correlate the evidence of phagocytosis in the liver with the lesion described in the lymph-nodes during the early (forty-eight hour) period. Definite evidence, however, of phagocytosis has been found in four animals, representing periods of one, two, eight, and nine days after the administration of serum, and representing, respectively, periods of three,²²⁵ sixty-five, and six days

after splenectomy. Also in a fifth animal, twenty-five days after splenectomy and fifteen days after the administration of the serum, the cells of the capillaries contained small balls of yellow pigment, apparently representing altered haemoglobin.

As controls we have examined the livers of several normal dogs and of nine splenectomized dogs not receiving serum, but without finding evidence of phagocytosis on the part of the cells of the liver capillaries, or of proliferation of these cells. The splenectomies in this series represented periods of from three to eighty-four days; five under ten days and three over twenty days.

Likewise we have examined the livers of nine normal dogs receiving haemolytic serum. In two of these the endothelial cells appeared to be increased somewhat in number, but no undoubted evidence of phagocytosis could be obtained. All other livers examined showed no changes in the cells of the capillaries.

MICROCHEMICAL TESTS FOR IRON IN LYMPH-NODES AND LIVER

Warthin, in his study of the lymph-nodes and haemolymph-nodes of sheep and goats, found in these organs, several months after splenectomy, evidence of increased blood destruction. This we have observed in the dog, in so far as it is shown by phagocytosis of red cells, only after the administration of a haemolytic poison; in the ordinary course of events, no increased phagocytic activity on the part of the endothelial cells of the lymph-nodes of the splenectomized animal is seen. This observation we have confirmed by examining a number of lymph-nodes and liver by the usual microchemical method of demonstrating the presence of iron. The lymph-nodes of fourteen sple-

nectomized dogs showed a considerable amount of iron in three, slight amounts in five, and none in six. The animals examined represented periods of eleven days to twenty-two months after splenectomy. In the lymph-nodes of fifteen normal animals similarly examined moderate amounts of iron were found in eight, slight amounts three times, and in four none. It is evident, therefore, that in the dog the iron content of the lymph-nodes after splenectomy differs little from normal. The liver likewise shows no increased deposition of iron. Of fourteen livers from splenectomized dogs, four showed slight deposition of iron in Kupffer's cells, while ten showed none. At the same time the livers of six normal dogs were similarly examined; in three slight deposits of iron were found, and in three none.

Protocols illustrating the histological changes in the lymph-nodes and liver follow:

Dog 32.—Splenectomy was performed under ether anaesthesia on July 19, 1911; on March 8, 1912, specific haemolytic immune serum was injected intravenously. The red cells dropped within three hours from 6,120,000 to 5,200,000 per cubic millimetre, and the haemoglobin, after twenty hours, to 42 per cent. Haemoglobinuria was present, and death occurred after forty-eight hours.

HISTOLOGY.—The liver-cells are pale, granular, stain poorly, and present here and there small areas of focal necrosis. The capillaries are dilated and contain much granular material and, as seen by the low power, numerous isolated round and oval clumps of red blood-corpuscles. By higher power of the microscope these clumps of red cells are found to be, in large part, within endothelial cells (Plate I, Fig. 4). Some of the red cells stain well with eosin, others appear as shadows. Other endothelial cells

are seen which contain mere fragments of red cells or masses of granular, yellow pigment, or large, yellow hyaline balls of apparently fused red cells. Attempts to demonstrate similar phagocytic cells in the large vessels of the liver and of other organs failed; they were present, however, in the sinuses of the lymph-nodes.

Dog 34.—Splenectomy was performed under ether anaesthesia on March 11, and haemolytic serum administered intravenously on March 14. Death occurred on March 15, after reduction of red cells to 1,960,000 and haemoglobin to 57 per cent. Haemoglobinuria was marked.

HISTOLOGY.—A mesenteric lymph-node shows hemorrhage, oedema, and extensive infiltration with polymorphonuclear leucocytes. The sinuses, both peripheral and central, are closely packed with large, pale, endothelial cells, nearly all of which contain red blood-cells, a single high-power field showing thirty to forty phagocytic cells (Plate I, Figs. 1 and 2). The number of engulfed red blood-cells varies, but is usually large, ten to twenty not infrequently being found in a single cell. In many endothelial cells, on the other hand, the red blood-cells have fused to form large, round, or oval hyaline masses still staining deeply with eosin. Between the phagocytic cells is much granular, eosin-staining material suggesting disintegrated red cells, mingled with serum, through which run irregular threads of fibrin. Here and there in the follicles are small areas of necrosis. Moderate leucocytic infiltration is seen throughout the section. Phagocytic cells cannot be demonstrated in the blood-vessels or in a tangle of lymphatic vessels present at one side of the node.

Other lymph-nodes (gastrohepatic, prevertebral, and bronchial) present the same lesions.

The liver of this animal showed widespread necrosis, but in the non-necrotic areas phagocytic endothelial cells are found in the capillaries (Plate I, Fig. 3).

SUMMARY

In a large proportion of dogs that have been splenectomized for periods of two weeks or more one finds a great increase in the number of endothelial cells of the lymph-nodes. In most splenectomized dogs that succumb to an injection of haemolytic immune serum within forty-eight hours the sinuses of the lymph-nodes contain large numbers of endothelial cells, phagocytic for red blood-cells. This is not seen in normal dogs receiving haemolytic serum. Likewise a similar power of phagocytosis is seen frequently in the stellate cells (Kupffer's) of the capillaries of the liver. Both in the lymph-nodes and the liver these cells appear to be formed *in situ*; we find no evidence that they have been transported to these organs.

Such findings suggest the development of a compensatory function on the part of the lymph-nodes and possibly the liver. Normally the spleen contains cells which have the power to engulf and presumably to destroy the red blood-corpuscles. In certain pathological conditions this function is frequently greatly augmented and may sometimes be shared by the lymph-nodes; for example, in typhoid fever, as was first clearly shown by Mallory.²⁷⁰ Our observations suggest that in the absence of the spleen this function of forming red blood-corpuscle phagocytic cells, normally a minor activity of the lymph-nodes, becomes highly developed in the latter organs, and that in times of stress these cells and the stellate cells of the liver thus assume, in part at least, the function of destroying red blood-corpuscles by phagocytosis.

CHAPTER VIII

METABOLISM STUDIES ON THE DOG BEFORE AND AFTER SPLENECTOMY

ALTHOUGH early in our work we studied the influence of splenectomy upon iron metabolism (see p. 112), it did not at that time seem advisable, on account of the generally negative results of others, to study the effect of splenectomy upon nitrogen metabolism. Later, however, when interested in the influence of diet in connection with the anaemia of splenectomy, we became aware of the observations of Richet,³⁷⁴ which seemed to indicate that in order to maintain the weight of a splenectomized dog a much larger amount of food is required than is the case with the normal dog.

These studies suggested to us a possible explanation of the contradictory and confusing results obtained in our dietary studies, and, as nitrogen metabolism had never been studied in animals in nitrogen equilibrium, we undertook a detailed investigation in the hope of arriving at some definite conclusion concerning (1) the influence upon metabolism of the absence of the spleen, as contrasted with (2) the influence on metabolism of the anaemia which usually follows splenectomy.

PREVIOUS INVESTIGATIONS

Paton's³²⁹ investigation included studies of the nitrogen metabolism and the elimination of salts in a single

dog before and after splenectomy. Observations were made during fasting and on (1) meat, (2) oatmeal and milk, and (3) rich nuclein diets. The first post-splenectomy metabolism study was made twenty-six days and the last four months after the operation. Paton's general conclusion is that under the various conditions of his experiments splenectomy causes no essential difference in the course or nature of the metabolism.

In Richet's first investigation³⁷⁴ nine splenectomized dogs were contrasted with six normal dogs. No metabolism studies and no examinations of the blood were made. Conclusions were based on records of food taken and the weight of the animals at various intervals. The increased consumption of food by the splenectomized animals is thought by Richet to be due to an increased catabolism in those animals and not to any disturbance of digestion. In a later report³⁷⁴ he refers to studies of seventeen splenectomized dogs, of which five were under observation for about two years, and confirms the conclusions of his earlier report. In this connection it is a matter of importance that the conclusions are based on the averages of two groups of dogs of widely different weights. Richet has not contrasted splenectomized dogs of given weight with normal dogs of the same weight, but if one selects from his tables dogs of the same weight the differences in food consumption are found to be very slight. Only two dogs were studied both before and after splenectomy.

Mendel and Jackson,²⁸⁵ who investigated the relation of the spleen to purin metabolism, found that in splenectomized dogs and cats no changes occurred.

Verzár⁴⁵² has found that extirpation of the spleen in dogs has no appreciable effect upon the respiratory gas

exchange. A similar conclusion was reached by Korenchevski²²⁴ as regards both gaseous and nitrogenous metabolism. No other experimental studies of the influence of splenectomy are available, except the brief note of Austin and Ringer²¹ to the effect that in the dog the absence of the spleen does not in any way modify the course of the glycosuria caused by pholorhizin.

METHODS

The four dogs used in this study were placed upon a constant diet of beef (usually beef-heart), lard, and sugar, the amounts of each of which constituents varied according to the caloric needs of each dog. The standard diet contained 0.4 gm. of nitrogen per kilo. and 70 calories per kilo. of body weight. A small amount of sodium chloride was given each day, and a sufficient amount of bone-ash was added to ensure well-formed fæces. The water intake for each day was constant. To some animals the beef-heart was given raw; in other instances it was boiled. After one or two weeks on the special diet, if the weight of the animal remained constant, a preliminary metabolism study, covering a period of seven days, was made. If the results of this were satisfactory, the animal was then splenectomized and the metabolism studies resumed at various intervals after the operation. In each experiment the diet after operation was always the same as before, and was continued without change in the intervals between periods of metabolism study. Analyses were made of all foods for fat and total nitrogen. During the periods of study the animals were kept in the usual metabolism cages. They were catheterized at the end of every twenty-four hours and the fæces marked by carmine.

In the analysis of the urine the total nitrogen was determined by the Kjeldahl-Gunning method, ammonia by Folin's method,¹²⁰ creatine and creatinine by Folin's method,¹²¹ and the hydrogen ion concentration according to Henderson's technique.¹⁷³ In the study of faeces the Kjeldahl-Gunning method was used for total nitrogen, the Folin-Wentworth method for fat,¹²² and Neumann's method³¹⁵ for iron.

The removal of the spleen, an essentially bloodless operation, was done under ether anaesthesia.

RESULTS

The details of our studies of nitrogen metabolism are shown in Tables XLVIII to LI, and of fat metabolism in Table LII.

Nitrogen Metabolism.—Table XLVIII represents the earliest period of metabolic study (three days) after splenectomy. The animal showed no loss of weight, no ill-effect of the operation, and the conditions were therefore ideal for the detection of any slight early changes in metabolism which might be due to the absence of the spleen. No variations in nitrogen partition were observed, however, and the nitrogen equilibrium was maintained: an average daily balance before operation of 0.45 gm. and after operation of 0.46 gm.

Table XLIX shows practically the same results, thirteen days and eight weeks after splenectomy. The animal was in nitrogen equilibrium before splenectomy, and maintained that condition after splenectomy. The general metabolism shows entirely normal results. The utilization of nitrogen was in no way interfered with: it was 94 per

cent. before operation and 95 and 93 per cent. in the post-splenectomy periods.

In Table L, which presents observations two, six, and ten weeks after splenectomy, the results in the third and fourth periods (sixth and tenth weeks) are similar to those

TABLE XLVIII

DOG 57. NITROGEN METABOLISM BEFORE AND THREE DAYS AFTER SPLENECTOMY

Date	Urine											N balance
	Weight kg.	N intake *	Amount cc.	Specific gravity	Reaction to litmus	H ion con- centration†	Total N gm.	Ammonia N gm.	Creatinine gm.	Creatine gm.	Faeces, total N gm.	
Feb. 28	10.6	4.80	295	29	Acid	6.90	3.45	0.13	0.289	0.478	0.73	4.18 +0.62
Mar. 1	10.8	4.80	175	34	Acid	6.50	3.76	0.15	0.270	0.357	0.73	4.09 +0.71
Mar. 2	10.8	4.80	225	37	Acid	6.90	3.44	0.12	0.289	0.326	0.73	4.17 +0.63
Mar. 3	10.8	4.80	225	38	Acid	6.90	3.57	0.13	0.285	0.321	0.73	4.30 +0.50
Mar. 4	10.8	4.80	190	37	Acid	6.90	3.65	0.14	0.289	0.335	0.73	4.38 +0.42
Mar. 5	10.9	4.80	200	40	Acid	6.90	3.86	0.14	0.289	0.335	0.73	4.59 +0.21
Mar. 6	10.9	4.80	220	27	Acid	6.90	4.02	0.12	0.279	0.377	0.73	4.75 +0.05
Average .	10.8	4.80	219	35		6.84	3.62	0.13	0.284	0.361	0.73	4.35 +0.45
Mar. 7	Splenectomy											
Mar. 10	10.8	4.80	150	45	Acid	6.15	3.86	0.18	0.311	0.310	0.53	4.39 +0.41
Mar. 11	10.8	4.80	220	35	Acid	6.50	3.92	0.15	0.311	0.310	0.53	4.45 +0.35
Mar. 12	10.9	4.80	235	37	Acid	6.50	3.96	0.14	0.332	0.285	0.53	4.49 +0.31
Mar. 13	10.9	4.80	180	40	Acid	6.50	4.08	0.13	0.302	0.320	0.53	4.61 +0.19
Mar. 14	10.9	4.80	135	45	Acid	6.15	3.95	0.15	0.289	0.267	0.53	4.48 +0.32
Mar. 15	10.9	4.80	165	37	Acid	6.15	3.36	0.13	0.289	0.259	0.53	3.89 +0.91
Mar. 16	11.0	4.80	210	42	Acid	6.90	3.53	0.08	0.311	0.283	0.53	4.06 +0.74
Average .	10.9	4.80	185	40		6.41	3.81	0.16	0.306	0.291	0.53	4.34 +0.46

* Diet: raw beef, 150 gm.; lard, 50 gm.; sugar, 50 gm.

† Expressed as negative logarithms.

shown in Tables XLVIII and XLIX. In the early period after splenectomy, however, this animal showed a loss of appetite which caused, during the two weeks following operation, a loss in weight of 1.4 kilos. This loss of appetite was not due to infection or other post-operative dis-

turbances, but appeared to be due rather to a dislike of the lard in the diet. When the lard was cut out of the diet the animal ate readily, and later, when the lard was again added, no trouble was experienced. As may be seen in Table LII, this was the only animal which showed a high

TABLE XLIX

DOG 48. NITROGEN METABOLISM BEFORE AND TWO AND EIGHT WEEKS AFTER SPLENECTOMY

Date	Weight kg.	N intake *	Amount cc.	Specific gravity ^r	Urine						Faeces, total N N of urine and faeces	N balance	
					Reaction to litmus	H ion con- centration†	Total N gm.	Ammonia N gm.	Creatinine gm.	Creatine gm.			
Nov. 16	13.4	5.60	175	56	Acid	6.70	5.35	0.38	0.300	0.358	0.34	5.69	-0.09
Nov. 17	13.4	5.60	250	47	Acid	6.90	5.48	0.37	0.338	0.195	0.34	5.82	-0.22
Nov. 18	13.4	5.60	225	50	Acid	6.70	4.91	0.38	0.352	0.480	0.34	5.25	+0.35
Nov. 19	13.4	5.60	210	43	Acid	6.80	5.33	0.37	0.368	0.427	0.34	5.67	-0.07
Nov. 20	13.4	5.60	200	44	Acid	6.90	4.80	0.34	0.361	0.467	0.34	5.14	+0.46
Nov. 21	13.4	5.60	235	46	Acid	6.90	4.91	0.31	0.368	0.408	0.34	5.25	+0.35
Nov. 22	13.3	5.60	265	37	Acid	6.90	4.77	0.27	0.385	0.597	0.34	5.11	+0.49
Average .	13.4	5.60	223	46		6.83	5.08	0.35	0.353	0.419	0.34	5.42	+0.18
Nov. 24													
Splenectomy													
Dec. 7	13.2	5.60	195	55	Acid	7.14	5.19	0.31	0.340	0.575	0.30	5.49	+0.11
Dec. 8	13.1	5.60	280	35	Acid	6.00	5.36	0.34	0.368	0.513	0.30	5.66	-0.06
Dec. 9	13.1	5.60	290	33	Acid	6.90	4.95	0.29	0.352	0.636	0.30	5.25	+0.35
Dec. 10	13.1	5.60	265	39	Acid	6.80	4.65	0.29	0.368	0.513	0.30	4.96	+0.64
Dec. 11	13.1	5.60	235	38	Acid	6.80	4.38	0.29	0.324	0.527	0.30	4.68	+0.92
Dec. 12	13.1	5.60	295	36	Acid	6.80	4.40	0.27	0.329	0.558	0.30	4.70	+0.90
Dec. 13	13.1	5.60	245	35	Acid	6.80	4.52	0.23	0.385	0.672	0.30	4.82	+0.78
Average .	13.1	5.60	256	39		6.75	4.78	0.29	0.352	0.570	0.30	5.08	+0.51
Jan. 18	13.6	5.70	275	30	Acid	6.80	4.46	0.22	0.355	0.398	0.40	4.86	+0.84
Jan. 19	13.6	5.70	210	44	Acid	6.80	4.32	0.24	0.346	0.382	0.40	4.72	+0.98
Jan. 20	13.6	5.70	310	20	Acid	6.70	4.58	0.22	0.364	0.361	0.40	4.98	+0.72
Jan. 21	13.5	5.70	300	39	Acid	6.80	4.92	0.21	0.355	0.474	0.40	5.32	+0.38
Jan. 22	13.5	5.70	250	35	Acid	6.70	4.49	0.24	0.311	0.362	0.40	4.89	+0.81
Jan. 23	13.4	5.70	300	39	Acid	6.90	5.12	0.23	0.337	0.463	0.40	5.52	+0.18
Jan. 24	13.4	5.70	300	39	Acid	6.90	5.51	0.22	0.326	0.405	0.40	5.91	-0.21
Average .	13.5	5.70	278	35		6.80	4.77	0.22	0.342	0.406	0.40	5.47	+0.53

* Diet: raw beef heart, 200 gm.; lard, 60 gm.; sugar, 60 gm.

† Expressed as negative logarithms.

neutral fat content in the faeces, though what relation there may be between this and the dislike of fat is not evident. The practical result of this loss of weight after splenectomy was a moderate retention of nitrogen in the first post-splenectomy metabolism period. However, in the third period, when the animal had returned to exactly the same weight as before operation, nitrogen equilibrium was again maintained. It would seem conclusive, therefore, that the loss of weight and nitrogen retention of the earlier periods were due to an influence other than the absence of the spleen. It is of interest that this dog excreted no creatine.

In the experiments thus far presented there is no evidence that the absence of the spleen influences in any way nitrogen metabolism. In a fourth animal, however, the results were discordant.

This animal (Table LI) had served as a control for the blood counts of the three animals discussed above, and up to the time of our foreperiod had been for twelve weeks on an adequate constant diet, as was the case in the other animals. Like Dog 52, this animal received boiled meat as a part of the dietary. The effect of splenectomy on the nitrogen metabolism, ten days after the operation, was very slight, but a nitrogen equilibrium of +0.48 gm. per day was changed to one of —0.18 gm., figures not beyond the range of normal variations, but which, in the light of changes to be discussed later, are suggestive of the influence of anaemia. At a later period, three months after splenectomy, the animal had not regained the slight loss (0.5 kilo.) in weight, but it appeared to be in excellent condition and the anaemia, which had existed for several months, was improving. The plus balance of 1.10 gm. of nitrogen per day (upon a slightly higher nitrogen intake)

TABLE L

**DOG 52. NITROGEN METABOLISM BEFORE AND TWO, SIX, AND TEN WEEKS
AFTER SPLENECTOMY**

Date	Urine												N balance	
	Weight kg.	N intake *	Amount cc.	Specific gravity	Reaction to litmus	H ion con- centration†	Total N		Ammonia N		Creatinine	Creatine	Faeces, total N gm.	N of urine and faeces
							gm.	gm.	gm.	gm.				
Nov. 25	10.8	4.77	170	44	Acid	6.50	4.17	0.30	0.368	None	0.38	4.58	+0.22	
Nov. 26	10.8	4.77	160	44	Acid	6.50	4.23	0.26	0.378	None	0.38	4.61	+0.16	
Nov. 27	10.8	4.77	225	33	Acid	6.50	4.49	0.23	0.368	None	0.38	4.87	-0.10	
Nov. 28	10.8	4.77	170	58	Acid	6.50	4.55	0.24	0.368	None	0.38	4.93	-0.16	
Nov. 29	10.8	4.77	180	46	Acid	6.50	4.55	0.25	0.368	None	0.38	4.93	-0.16	
Nov. 30	10.8	4.77	215	40	Acid	6.50	4.68	0.25	0.358	None	0.38	5.06	-0.22	
Dec. 1	10.8	4.77	160	43	Acid	6.50	4.44	0.25	0.351	None	0.38	4.82	-0.05	
Average .	10.8	4.77	183	44		6.50	4.44	0.25	0.365		0.38	4.82	-0.05	
Dec. 2	Splenectomy													
Dec. 15	9.4	4.70	225	23	Acid	6.90	2.75	0.16	0.213	None	0.44	3.19	+1.51	
Dec. 16	9.4	4.70	180	39	Acid	6.50	3.73	0.25	0.324	None	0.44	4.17	+0.55	
Dec. 17	9.5	4.70	115	43	Acid	6.80	2.99	0.15	0.281	None	0.44	3.43	+1.27	
Dec. 18	9.5	4.70	210	35	Acid	6.80	2.79	0.15	0.289	None	0.44	3.23	+1.47	
Dec. 19	9.6	4.70	85	53	Acid	6.50	2.48	0.20	0.295	None	0.44	2.92	+1.78	
Dec. 20	9.7	4.70	275	20	Acid	6.70	2.79	0.25	0.291	None	0.44	3.23	+1.47	
Dec. 21	9.8	4.70	175	30	Acid	6.70	2.52	0.23	0.289	None	0.44	2.96	+1.74	
Dec. 22	9.8	4.70	225	25	Acid	6.80	2.88	0.25	0.311	None	0.44	3.32	+1.38	
Average .	9.6	4.70	186	34		6.71	2.87	0.21	0.287		0.44	3.31	+1.39	
Jan. 12	10.3	4.40	235	28	Acid	6.00	3.33	0.27	0.311	None	0.48	3.81	+0.59	
Jan. 13	10.3	4.40	190	35	Acid	6.30	3.38	0.24	0.311	None	0.48	3.86	+0.54	
Jan. 14	10.3	4.40	210	35	Acid	6.15	3.60	0.26	0.311	None	0.48	4.08	+0.32	
Jan. 15	10.4	4.40	180	42	Acid	6.30	3.45	0.22	0.324	None	0.48	3.93	+0.47	
Jan. 16	10.4	4.40	175	39	Acid	6.30	3.54	0.25	0.324	None	0.48	4.02	+0.38	
Jan. 17	10.4	4.40	225	35	Acid	6.70	3.38	0.25	0.311	None	0.48	3.86	+0.54	
Jan. 18	10.4	4.40	205	29	Acid	6.30	3.57	0.26	0.311	None	0.48	4.05	+0.35	
Average .	10.35	4.40	203	35		6.29	3.46	0.25	0.318		0.48	3.94	+0.45	
Feb. 10	10.8	4.10	160	35	Acid	6.90	2.96	0.23	0.225	None	0.44	3.40	+0.70	
Feb. 11	10.8	4.10	215	25	Acid	6.80	3.11	0.25	0.228	None	0.44	3.55	+0.55	
Feb. 12	10.8	4.10	220	22	Acid	6.80	3.23	0.20	0.234	None	0.44	3.67	+0.43	
Feb. 13	10.8	4.10	200	27	Acid	6.80	3.20	0.19	0.234	None	0.44	3.64	+0.46	
Feb. 14	10.9	4.10	190	32	Acid	6.80	3.44	0.18	0.231	None	0.44	3.88	+0.22	
Average .	10.8	4.10	197	28		6.82	3.19	0.21	0.230		0.44	3.63	+0.47	

* Diet: boiled beef heart, 100 gm.; lard, 50 gm.; sugar, 50 gm.

[†] Expressed as negative logarithms.

in this period, without change in weight, suggests the possibility of the utilization of this nitrogen for the repair of the anaemia. Utilization of protein was not disturbed,

TABLE LI

DOG 56. NITROGEN METABOLISM BEFORE AND TEN DAYS AND THREE MONTHS AFTER SPLENECTOMY

Date	Weight kg.	N intake * cc.	Urine.								Faeces, total N N of urine and faeces	N balance	
			Amount 10	Specific gravity	Reaction to litmus	II ion con- centration†	Total N gm.	Ammonia N gm.	Creatinine gm.	Creatine gm.			
Feb. 16	8.5	3.43	100	39	Acid	6.30	2.63	0.13	0.221	0.014	0.35	2.98	+0.45
Feb. 17	8.5	3.43	115	44	Acid	6.00	2.61	0.17	0.270	0.022	0.35	2.96	+0.47
Feb. 18	8.5	3.43	140	38	Acid	6.13	2.39	0.14	0.279	0.022	0.35	2.74	+0.66
Feb. 19	8.4	3.43	120	42	Acid	6.30	2.63	0.17	0.319	0.057	0.35	2.98	+0.45
Feb. 20	8.4	3.43	120	47	Acid	6.30	2.73	0.16	0.261	0.116	0.35	3.08	+0.35
Feb. 21	8.4	3.43	200	39	Acid	6.30	2.61	0.13	0.265	0.084	0.35	2.96	+0.47
Feb. 22	8.4	3.43	90	50	Acid	6.15	2.60	0.14	0.253	0.067	0.35	2.95	+0.48
Average.	8.4	3.43	126	43		6.21	2.60	0.15	0.267	0.055	0.35	2.95	+0.48
Feb. 23					Splenectomy								
Mar. 5	8.0	3.37	165	47	Acid	6.15	3.11	0.13	0.231	0.121	0.41	3.52	-0.15
Mar. 6	8.0	3.37	110	46	Acid	6.15	3.00	0.14	0.213	0.143	0.41	3.41	-0.04
Mar. 7	8.0	3.37	150	44	Acid	6.50	3.19	0.14	0.234	0.081	0.41	3.60	-0.04
Mar. 8	8.0	3.37	210	40	Acid	6.15	3.30	0.16	0.221	0.119	0.41	3.71	-0.34
Mar. 9	7.9	3.37	170	42	Acid	6.15	3.32	0.16	0.213	0.144	0.41	3.73	-0.36
Mar. 10	7.9	3.37	130	49	Acid	5.85	3.18	0.16	0.213	0.114	0.41	3.59	-0.22
Mar. 11	7.9	3.37	170	38	Acid	6.15	3.09	0.12	0.213	0.129	0.41	3.50	-0.13
Average.	8.0	3.37	152	44		6.14	3.17	0.14	0.220	0.122	0.41	3.72	-0.18
May 20	7.9	3.78	130		Acid	2.17			0.213	0.029	0.38	2.55	+1.23
May 21	7.9	3.78	145		Acid	2.07			0.213	0.014	0.38	2.45	+1.33
May 22	7.9	3.78	130		Acid	2.24			0.225	0.010	0.38	2.62	+1.16
May 23	7.9	3.78	260		Acid	2.56			0.238	0.017	0.38	2.94	+0.84
May 24	7.9	3.78	115		Acid	2.47			0.213	0.014	0.38	2.85	+0.93
Average.	7.9	3.78	156			2.30			0.220	0.017	0.38	2.68	+1.10

* Diet: boiled beef heart, 75 gm.; lard, 40 gm.; sugar, 40 gm.

† Expressed as negative logarithms.

being 90 per cent. in Period I, 88 per cent. in Period II, and 90 per cent. in Period III.

Unlike the other three dogs, we had here in Period II an increase of creatine amounting to 45 per cent. This

increase was at the expense of the creatinine, however, for the total creatinine, including preformed creatinine and creatine as creatinine, agrees very closely, in the two periods, amounting to 0.314 gm. in the foreperiod and 0.325 gm. in the after-period.

TABLE LII
FAT DETERMINATIONS BEFORE AND AFTER SPLENECTOMY

Dog No.	Period *	Total intake	Total output	Fat utilized	Total output of fatty acids including soaps	Fatty acids in total output	Total output neutral fats	Neutral fat in total output
48	I (7 days)	gm. 460.6	gm. 26.54	per cent. 94.2	gm. 22.45	per cent. 84.4	gm. 4.09	per cent. 15.6
	Nov. 24	Splenectomy						
	II (7 days)	460.6	20.92	95.5	18.11	86.5	2.81	13.5
52	III (7 days)	460.6	15.77	96.6	11.51	73.0	4.26	27.0
	I (7 days)	374.8	9.19	97.5	6.29	68.4	2.90	31.6
	Dec. 2	Splenectomy						
	II (8 days)	428.32	12.64	97.0	5.93	46.9	6.71	53.1
56	III (7 days)	374.8	17.14	95.4	8.44	49.2	8.70	50.8
	IV (5 days)	267.7	7.85	97.1	6.71	85.5	1.14	14.5
	I (7 days)	298.4	13.59	95.4	9.27	68.3	4.32	31.7
57	Feb. 23	Splenectomy						
	II (7 days)	298.4	14.25	95.2	11.11	78.0	3.14	22.0
57	I (7 days)	380.45	23.87	93.7	19.42	81.4	5.04	18.6
	Mar. 7	Splenectomy						
	II (7 days)	380.45	10.56	97.2	7.68	72.7	2.88	27.3

* These periods correspond exactly to those in Tables I, II, III, and IV.

During Period III, while the average creatine output was exactly the same as during Period II, the creatine output fell to a figure lower than either of the preceding periods. The variation in the partition of creatine and creatinine in this animal we are unable to explain.

Fat Utilization.—The utilization of fat (Table LII) in all the animals was normal in all periods. The partition of fatty acids (including soaps) and neutral fats shows some variation, especially in Dog 52, but to this we are inclined to ascribe no importance. A thorough search of the literature shows that no studies of fat utilization in animals before and after splenectomy have previously been made.

Iron Metabolism.—As has been shown elsewhere (see p. 119), Dogs 57, 48, and 52 showed no important change in the elimination of iron after splenectomy, while Dog 56 did.

DISCUSSION

These observations show that in three of four animals the removal of the spleen had no effect upon nitrogen

TABLE LIII
BLOOD EXAMINATIONS

Dog No.	Period *	Hæmoglobin <i>per cent.</i>	Red cell count
48	I	100	5,900,000
	II	104	5,570,000
	III	100	5,540,000
52	I	105	6,700,000
	II	106	6,840,000
	III	84	5,360,000
	IV	90	5,100,000
56	Initial	105	6,450,000
	I	83	6,020,000
	II	70	5,890,000
	III	72	4,950,000
57	I	95	6,130,000
	II	90	6,130,000

* The periods correspond exactly to those of the previous tables.

metabolism, the utilization of fat, or the elimination of iron, and justifies the conclusion that the removal of the

normal spleen in a normal animal has no important effect upon general metabolism. It is necessary, however, in order that there may be no question about this conclusion, to explain the discordant results in the fourth animal (Dog 56). This animal showed a loss of weight, an increased elimination of iron (see p. 119), and a disturbance of creatine metabolism. The fat metabolism was unaltered. The question arises whether these changes are due to the absence of the spleen or to the anæmia which was present. In Table LIII are presented the blood examinations of each dog at the time of the several metabolism periods.

It will be seen that Dogs 48 and 57 showed no appreciable change in the blood picture after splenectomy, but that Dogs 52 and 56 did. The blood changes in Dog 52, however, were relatively slight. The situation in regard to Dog 56 was somewhat different. This animal had been placed on a constant diet, the chief article of which was boiled beef-heart, twelve weeks before the first metabolism study. At that time the blood examination showed haemoglobin 105 per cent. and red cells 6,450,000. At the time of our presplenectomy period it showed a relatively low haemoglobin content (83 per cent.) and 6,020,000 red cells. After splenectomy the haemoglobin continued to fall until, two and a half months after operation, it showed the low level of haemoglobin 60 per cent. and red cells 4,560,000. It is evident, therefore, that this animal differed from the other three in that it developed an anæmia more rapidly and eventually of a more severe grade than was the case in any other animal of this series. As we have shown elsewhere, anæmia of varying severity is a fairly constant result of splenectomy in the dog. The anæmia may be slight, as in Dog 52, or more severe, as in Dog 56, and may, as

has been suggested, be lessened by diet. The influence of diet we have already discussed, but it is not a matter which concerns us at the present time (see p. 22). The essential fact is that Dog 56 developed a severe anaemia, already progressive at the time of the first metabolism study, while Dogs 48 and 57 were not anaemic, and Dog 52 showed only a slight non-progressive deterioration of the blood. The question naturally arises: Is the increased elimination of iron and the disturbance of the creatine metabolism due to the anaemia and not to an influence on metabolism consequent upon the absence of the spleen?

A few words are necessary concerning Richet's statement that the splenectomized dog requires more food to maintain its weight than does the normal dog. In view of our results, Richet's conclusion is not tenable. Dog 57 (Table XLVIII) maintained its presplenectomy weight without change in diet and with only a slight change in the nitrogen balance. Dog 48 (Table XLIX) likewise showed only a trifling change during the three weeks after operation and a return to the previous weight after seven to eight weeks. The serious loss of weight in Dog 52 was due to loss of appetite, and that, relatively slight, in Dog 56 was complicated by the coexisting anaemia.

Moreover, during the past five years we have frequently noticed a tendency for splenectomized dogs to become obese, and this tendency is mentioned also by several investigators who have studied splenectomized animals for long periods of time. This tendency to put on weight is strikingly shown by two of the dogs (48 and 52) of this series. At the close of the metabolism work, presented in Tables XLIX and L, these animals were not destroyed, on account of the possible necessity of repeating

the metabolism studies after longer periods had elapsed. The change from a special to the ordinary kennel diet ("table scraps") led to a rapid increase of weight in each instance; in three months the weight of Dog 48 increased from 13.4 to 15.8 kilos., while in two months Dog 52 rose from 10.9 to 12.9 kilos.

Our results are therefore in accord with those of Paton rather than with those of Richet, and demonstrate that in the absence of anaemia the removal of the spleen has no influence upon nitrogen or fat metabolism, and in all probability no influence upon iron elimination.

Our general results may be summarized as follows:

Four dogs have been subjected to metabolism studies before splenectomy and at intervals of three days to three months after splenectomy. In three of the four animals the removal of the spleen was not followed by any disturbance of nitrogen metabolism, fat utilization, or iron elimination. Two of these animals showed no anaemia, and the third only a slight reduction in haemoglobin and number of red cells.

A fourth animal, studied ten days and three months after splenectomy, developed eventually a definitely progressive anaemia of moderate severity. This animal showed a slight loss of weight, a slight disturbance of nitrogen balance, and of creatine-creatinine partition, with a marked increase in the elimination of iron. We conclude, therefore, that, under the conditions of our experiments, there is no evidence that the spleen has an influence on metabolism, and we regard the disturbances occurring in one of our dogs as due to the coexisting anaemia and not to the absence of the spleen.

CHAPTER IX

GENERAL SUMMARY OF EXPERIMENTAL STUDIES

AFTER splenectomy in dogs three prominent phenomena are observed:

1. An anæmia of the secondary type, mild or moderate in character, which usually reaches its severest stage after one and a half months and is followed by repair, which is well advanced after three or four months but may not be complete for longer periods of time.
2. An increased resistance of the red blood-cells to hypotonic salt solutions, hæmolytic serum, saponin and cobra venom, and mechanical shaking.
3. A lessened tendency to hæmoglobinuria and jaundice, and sometimes an absence of jaundice, after the administration of hæmolytic agents.

The anæmia may be irregular in its onset and severity, as may be also the repair process, but as a rule it has a very definite course. The decrease in hæmoglobin content occurs usually a little later and is often slightly more marked than the decrease in red blood-cells. The former seldom falls below 55 per cent. or the latter below 3,000,-000. Neither during the period of anæmia nor repair do nucleated or other abnormal types of cell appear in the peripheral blood, except occasionally, at the time of beginning repair. The behavior of the white cells is quite constant; shortly after operation a marked rise to 26,000 to 38,000 occurs, with a return after a few days to 20,000 and a gradual decrease to normal level after one to four months. The increase in leucocytes is almost entirely an

increase in the polymorphonuclear type, the lymphocytes later showing a slight increase. The behavior of the eosinophiles is inconstant.

The "blood crisis," so frequently found after removal of the human spleen in splenic disease, is not present after the removal of the normal dog's spleen.

Dietary studies indicate that the anæmia is not influenced by the amount of iron furnished in the food. On the other hand, a small number of experiments furnish some evidence that the anæmia is apt to be more severe when the animal is fed on cooked food than when it is fed on an uncooked diet. Control experiments, in which other operative procedures, such as unilateral nephrectomy, have preceded splenectomy, prove conclusively that it is the absence of the spleen, and not post-operative accidents or complications, that is the essential factor in the production of anæmia.

A review of the literature of splenectomy in man shows that after removal of the spleen for injury or simple lesion not involving a disturbance of the function of the spleen, as rupture, twist, and cyst, a mild anæmia of variable course is the rule, and that in the absence of the spleen the repair of anæmia is delayed.

The increased resistance of the red blood-cells is a property of the red cell itself and is not due to an anti-hæmolytic power of the serum or to changes in complement content. The exact reason of this increased resistance is not evident. Its association with anæmia and the concomitant repair suggests that the presence of young and more resistant cells might explain it. We have not been able, however, to demonstrate an increase in nucleated cells or in the reticulated cells, usually considered as young forms indicative of active blood formation and more resist-

ant to lytic agents. We believe, however, that the increased resistance of the red cells is not dependent merely on the absence of the spleen, but is, in part at least, in some way associated with the anaemia, or the factor or factors causing it, thus differing, in our view, from Banti and his associates.

In regard to the decreased tendency to haemoglobinuria and jaundice after the administration of haemolytic agents, we offer experimental evidence to indicate that (1) absence of the spleen does not prevent the secretion of bile; (2) the spleen does not influence the transformation of free haemoglobin into bile-pigment, and (3) that fresh splenic extracts have no demonstrable action *in vitro*. Two important factors appear to be (*a*) the influence of anaemia, and (*b*) the increased resistance of the red cells, in that each reduces the amount of haemoglobin set free. The most important factor, and a purely mechanical one, concerns the way in which the haemoglobin set free by blood destruction reaches the liver. Under normal conditions the disintegrating blood-cells accumulate in the spleen and the liberated haemoglobin normally reaches the liver directly and in concentrated form through the portal vein, and, as a result, haemoglobinuria and jaundice are more apt to occur. In the absence of the spleen it reaches the liver through the general circulation (hepatic artery) more slowly and much diluted, and haemoglobinuria is therefore less frequent and jaundice is of less degree or entirely absent. Our experiments in which injections of haemoglobin into the mesenteric vein were contrasted with injections of haemoglobin into the femoral vein offer conclusive proof of the correctness of this view, as do also experiments in which, by means of an Eck fistula and by anastomosis of the splenic vein with the vena cava, the splenic blood was

diverted from the liver without removal of the spleen. Another important mechanical factor connected with the blood supply to the liver is that in splenectomized animals the total volume of the portal blood supply is considerably lessened. Further support of this view is found in the anæmia that followed ligation of the splenic veins and in the single experiment where the mesenteric vein was unwittingly transplanted into the vena cava.

Another factor which we have discussed in this connection is that concerning the influence of fatty acids as brought forth by Joannovics and Pick. Their views are supported by Eppinger and King, who find, after splenectomy in the dog, a decrease in the unsaturated fatty acids and an increase in the cholesterol content of the blood, changes which might well have an influence on the degree and velocity of haemolysis. Their observations, unfortunately, we cannot confirm.

In regard to the problem of the anæmia, detailed comparison of the arterial and venous blood of the spleen offers no evidence to indicate, by the methods used, that the spleen has an important rôle in blood formation, or, on the other hand, that it is appreciably active in blood destruction.

The injection, however, into normal dogs of fresh spleen extract does cause an evanescent but definite increase in haemoglobin and red-cell content, which is not caused by extracts of other organs. This result would appear to be brought about through a stimulating action on the bone-marrow. On the other hand, fresh beef spleen fed liberally to splenectomized dogs does not prevent the occurrence of anæmia.

If in splenectomized dogs which have recovered from the

anaemia following removal of the spleen a second anaemia is produced by administering some haemolytic agent, this anaemia, as shown by direct blood examination, runs a longer course and has a longer period of repair than is the case in the normal dog. Here we have an apparent paradox, for, as has been pointed out above, the red cells of the splenectomized animal are more resistant to haemolytic agents than are those of the normal dog, and theoretically one would expect a mild anaemia of shorter course with quick repair.

Our explanation of the paradox is that in the spleenless dog some factor is at work, due to the absence of the spleen, which prevents rapid blood regeneration. The normal dog, though suffering as great, or even a greater, blood destruction, has no fault in regeneration, and its blood returns quickly to normal. On the other hand, in the splenectomized dog the factor delaying regeneration operates after an acute anaemia, as it did originally after removal of the spleen, and therefore the anaemia often reaches a lower level and is repaired more slowly.

Experiments undertaken to show that this unknown factor might be a function of the spleen concerned in the utilization of iron for the manufacture of haemoglobin have given negative results. Occasionally, shortly after splenectomy, a slight increase in elimination of iron was observed, but we have found no evidence of disturbance of iron metabolism one, nine, and twenty months after splenectomy. The most marked disturbance of iron metabolism occurred in a dog with a moderately severe anaemia, and we believe that an increased elimination of iron is a manifestation of increased blood destruction and not directly dependent on the absence of the spleen.

Control studies, in which without removal of the spleen the splenic blood was diverted from the liver by means of an Eck fistula or an anastomosis of the splenic vein with the vena cava or ligation of the splenic vein, yielded results similar to those following splenectomy, but varying somewhat in degree or duration. The anæmia and the icterus, as also the slow repair of anæmia, under these circumstances did not differ greatly from the results following splenectomy. If the anæmia is due to the loss of a stimulating action on the bone-marrow, these experiments show that access to the liver is necessary for the activation of such stimulant. The increased resistance of the red cells, on the other hand, did not persist for so long a time as after splenectomy. The lessened tendency to jaundice, on account of the important mechanical factor involved, was quite similar to that following splenectomy. These observations emphasize the importance of the mechanical factor (the disturbance of the direct blood flow to the liver), and suggest, also, some functional relation of the spleen to the liver that is not yet fully understood.

Studies of the bone-marrow after splenectomy show that, as a rule, the fatty marrow of the long bone is transformed into red marrow. During the early months this change is slight or absent, but after six to twenty months it is fairly constant and complete. There is no evidence that this change is compensatory to the early anæmia caused by splenectomy or to an increased hæmolysis in the lymph nodes. It may be that it is a concomitant of the activity of the bone-marrow in taking over, in the absence of the spleen, the function of storing and elaborating the iron of old blood-pigment, but our observations do not fully support this hypothesis.

The lymph-nodes, after splenectomy, exhibit, as a rule, a great increase in the number of endothelial cells. In most splenectomized dogs that succumb to a haemolytic agent within forty-eight hours the sinuses of the lymph-nodes contain large numbers of endothelial cells filled with red blood-corpuscles. This is seen, also, to a less extent in the case of the stellate cells of the liver capillaries. This phenomenon has not been seen to the same extent in normal dogs receiving haemolytic serum. Such findings suggest that in the absence of the spleen the function of forming red-blood-cell-phagocytic cells—normally a minor function of the lymph-nodes—becomes highly developed in these structures and is shared also by the liver, and that in times of stress, as after excessive blood destruction, these organs assume, in part at least, the function of destroying red blood-cells by phagocytosis. The small iron content of the lymph-nodes and liver indicates, however, that under normal circumstances, in the splenectomized animal, no excessive destruction of this type occurs.

Detailed metabolic studies have demonstrated conclusively that the removal of the normal spleen in a normal animal has no effect on nitrogen, fat, or iron metabolism. When disturbance of nitrogen and iron metabolism occurs it is due, in all probability, to a coexistent anaemia, and not to the mere absence of the spleen.

In conclusion, we wish to state frankly that, while the experiments here described add to our knowledge of certain phases of the relation of the spleen to blood destruction and regeneration, we are still in doubt about the exact cause of (*a*) the anaemia and (*b*) the increased resistance of the red cells which so constantly follows splenectomy.

CHAPTER X

METABOLISM STUDIES ON MAN BEFORE AND AFTER SPLENECTOMY

STUDIES of metabolism after splenectomy in the normal dog, as has been shown in the preceding chapter, are essentially negative. In man the situation is entirely different. Splenectomy, for chronic disease at least, is done in the presence of an anaemia of more or less severity, and the alterations in metabolism before operation are largely those dependent on the changes in the blood. The improvement following splenectomy is to be regarded, therefore, not as dependent on the removal of a normal function of the spleen, but as dependent on the removal of a haemolytic or other toxic activity for which the altered physiology of the spleen is responsible. Metabolism studies in man, under such conditions, thus become valuable adjuvants to the blood examination in determining the type of splenic disease or anaemia in which favorable results from splenectomy may be expected.

As investigations of this type are comparatively new—to our knowledge only five studies of conditions both before and after splenectomy have been made—we present in considerable detail two carried out under our direction: one in connection with congenital haemolytic jaundice¹⁵⁰ and the other in connection with pernicious anaemia.³⁴²

IN CONGENITAL HEMOLYTIC JAUNDICE

The subject of the first of these was a child suffering from congenital haemolytic jaundice presenting the following history:

At birth, at term, the child weighed seven pounds, and is described as lacking the characteristic red color of the newly born. About twenty-four hours after birth the "alabaster whiteness" of the skin, which the mother described, changed to a mahogany brown, which lasted three months, gradually fading to a sallow pallor, which has persisted. At six months, when the child passed through an attack of pneumonia, it weighed only ten pounds. The general health was poor and gastro-intestinal disturbances frequent. In the fourteenth month the first severe anaemia, accompanied by dark-brown discolourations of the skin and preceded by protracted vomiting and diarrhoea, was observed. Two months later a similar attack occurred, with the new feature of marked oedema of the entire body. During these attacks the rectal temperature usually rose to 104 or 105. Periods of recrudescence and exacerbation followed one another until the child was two and one-half years old, when an unusually severe attack kept him in bed for five months. Vomiting and diarrhoea were severe, and hemorrhages from the nose and bowel were frequent and difficult to control. During the second month of this period a partial paralysis of the left side developed. At this time an injection of neosalvarsan was given, more for the haematinic action of the arsenic than with any suspicion of lues. Gradual improvement followed this treatment, and after the paralysis had disappeared, except for a residual spastic palsy of the left leg, the child enjoyed fairly good health. The Wassermann reaction, frequently repeated, has always been negative. During the eight months following this attack salvarsan was given five times by rectum. About the time he was five years old, and again seven months later, he lost the power of speech, was

more or less delirious, and complained of pain in his head. At three and one-half and again at four and one-half years he had an otitis media. His appetite has always been poor, never normal, and at times he refuses to eat. A tendency to localized œdema, especially of the face and of the hand, has been constantly noted. He tires easily, and frequently complains of this. Obstinate constipation has been the rule, and the fæces are described as dark or orange in color.

From the family history it is found that a sister was jaundiced for ten days and a brother for two days after birth, but these children, now fifteen and thirteen years of age respectively, have otherwise been in good health. The father is said to have had an enlarged spleen and offers a history of exposure to lues, of skin eruption, and chronic abscess of joint and ankle. The mother, three months before the birth of the subject of the present study, was paralyzed and suffered a separation of the pelvic bones. The delivery was under anaesthesia.

Laboratory Examinations.—An examination of the numerous clinical records which have accumulated shows that the urine offers nothing of unusual interest. The only positive finding is an occasionally slight trace of albumin. Tests for bile have always been negative, and urobilin tests have shown no increase of this substance. Examinations of the fæces indicate that food is well digested; tests for occult blood have been negative, and no parasites or ova have been found. No records of the early blood examinations have been preserved, except a brief record that at the time of the first severe attack (when fourteen months old) the haemoglobin fell to 24 per cent. and a diagnosis of pernicious anæmia was made. A number of counts made during the years 1913 and 1914 showed considerable varia-

tion in both haemoglobin (from 65 per cent. to 28 per cent.) and red blood-cells (4,180,000 to 1,360,000). The Brûlé test and tests for cross haemolysis with normal serum and cells were negative. The subsequent blood counts are summarized in Table LIV.

At the beginning (December 3, 1914) of our metabolism studies a complete examination of the child was made, and from the notes at that time the following abstract of positive findings has been prepared:

The boy is five and one-half years of age, 105.5 cm. in height, weighs 39 pounds, is well nourished and of good muscular development. His skin is slightly sallow, but there is no true jaundice. Over the vessels of the neck a systolic bruit is heard, and an occasional slight systolic whiff is heard at the apex of the heart. At the base of the heart the first sound is replaced by a systolic murmur of a blowing character, and the second sounds are accentuated. Normal sounds are heard at the tricuspid area. The liver is doubtfully palpable. The splenic dulness begins in the mid-axillary line at the sixth rib and extends down a little below the line of the umbilicus; its greatest length is 18 cm. and its greatest width about 9 cm. The spleen feels firm, smooth, and without distinct notches. The right lower extremity is normal; the left lower extremity normal as to thigh and leg, but the ankle and foot show a spastic paralysis.

The urine at this time was amber in color, with no gross sediment; the specific gravity was 1.029; tests for albumin, sugar, acetone, diacetic acid, indican, and bilirubin were negative. The test for urobilin gave a doubtful reaction; under the microscope some mucus was seen, but no cells, casts, or crystals. The blood count at this time

THE SPLEEN AND ANÆMIA

TABLE LIV
Blood Examination Before and After Splenectomy in Congenital Haemolytic Jaundice

Date	Hb. per cent.	Erythro- cytes	Morphology of erythrocytes	Resistance to sodium chlorid solution				Percentage					
				Retic- ulated eryth- rocytes per- centage	Partial haemo- lysis	Com- plete haemo- lysis	White blood cells	Polys.	Lym- phos.	Trans.	Monos.	Eosinos.	Basos.
4/13/14	60	3,820,000	Poikilocytosis; polychromatophilia; occasional normoblasts; anisocytosis	4	0.65	0.425	6,600	41	47	...	3	7	2
6/ 1/14	50	3,450,000	Same as above; no Howell-Jolly bodies	6.1	0.6	0.375	9,600	26	72	1	1
12/ 3/14	23	2,020,000	Same as above; no Howell-Jolly bodies	9	0.625	0.375	6,100	49	46	3	2
1/26/15	33	2,290,000	Same as above; no Howell-Jolly bodies	...	0.65	0.425	6,800	54	43	...	1	2	...
Splenectomy													
1/28/15													
2/ 3/15	38	3,570,000	Not noted	16,600	62	32	3	2	4	1
2/11/15	45	4,190,000	Morphology much improved; no erythroblasts	15,800	70	22	3	2	2	1
2/15/15	45	4,480,000	Same as above; normal in appearance; no Howell-Jolly bodies	1	0.525	0.35	9,000	44	45	7.5	10	1	1.6
3/ 9/15	75	4,710,000	Normal.....	14,100	56	27.5	6	2.5	2.5	1.6
4/ 9/15	70	5,070,000	Normal.....	11,200	41	43	4	5	6	1

is given in Table LIV, under the date of December 3, 1914.

The first metabolic period ran from the 4th to the 14th of December, and during this time there was little or no change in the patient's condition. Throughout the period there was a tendency to elevation of temperature, which, however, rose but once over 101. The child was not kept in bed, ate regularly, and required no medication or cathartic. At the end of the period he was discharged, to return after Christmas.

During the interval at home (December 14, 1914, to January 26, 1915) no noteworthy change in condition occurred. On January 26 the patient was readmitted to the University Hospital, service of Dr. Charles H. Frazier. Physical examination revealed nothing new; examination of the blood and urine showed but little change. On January 28 the spleen was removed by Dr. Frazier, under ether anaesthesia. The operation was uneventful and there was only a trifling loss of blood. At the time of the operation the coagulation time of the blood was found to be about four minutes, and tests for resistance of red cells showed beginning of haemolysis in 0.65 per cent. and complete haemolysis in 0.425 per cent. salt solution. Convalescence after the operation was satisfactory, and on February 4 the patient was transferred to the medical service, and the second period of metabolism study was started on February 5. At this time the child's weight was 40 pounds.

On the fourth day of metabolism study an attack of bronchitis caused a rise of temperature to 103, and for two or three days there was some loss of appetite, and on the ninth day a mild otitis media developed. Despite these disturbances, the metabolism study was continued until the period of ten days representing the eighth to eighteenth days after splenectomy was completed.

At the end of the period the child's weight was $38\frac{1}{2}$ pounds and he seemed considerably improved in color and strength. The appetite was good. The condition of the bowels demanded an occasional cathartic, which, however, never caused watery stools. Even before discharge on February 18, just three weeks after operation, it was evident that a marked improvement in the blood picture had occurred, both haemoglobin and red blood-cell count being double that obtained on the first admission.

After discharge the improvement continued steadily, with greatly increased appetite and strength. The skin lost its sallow hue and became normal in appearance. In the two months since operation the child has enjoyed uninterrupted good health, except for one attack of indigestion, the result of overfeeding. Two weeks previous to the last blood count given in the table a rather severe nasal hemorrhage occurred as a result of excoriations. The lowered haemoglobin in the last count is probably to be explained by this hemorrhage.

PATHOLOGIC DESCRIPTION OF THE SPLEEN.—The weight is 640 gm.; length, 18.3 cm.; width, 10.8 cm.; thickness, 3.8 at one end, increasing to 8.4 at opposite end. The organ is of uniform bluish-red color. The capsule is for the most part smooth, with a few fine adhesions at one pole, where there is also a small circumscribed area (1.5 cm. in diameter) of thickening. The vessels of the hilum are normal. No supernumerary spleens are seen.

On section a large amount of dark fluid blood escapes. The cut surface has a uniform smooth, glistening appearance of dull-red color. The Malpighian bodies are distinctly visible, but not so large as in the normal spleen. The trabeculae are not prominent. The consistence is

increased, it being almost impossible to rupture the spleen by pressure with the thumb. At one end (the larger) of the organ is a distinctly circumscribed, but not encapsulated, mass (3.5 cm. in diameter), spherical in shape, which shows no Malpighian bodies, but does present a few minute ochre-colored areas. This area is of the same color and consistence as the rest of the spleen. On section it bulges prominently above the cut surface. The weight of the spleen after escape of fluid blood from three longitudinal incisions is 435 gm.

Gross Diagnosis.—Splenomegaly with area of recent infarction.

Microscopic Appearance.—Very slight thickening of capsule with no increase of trabeculae. The sinuses are dilated and congested. The reticulum is increased in amount, and the cells of pulp appear to be decreased in number. The Malpighian bodies show no change except a hyaline thickening of central arteries which is evident in arteries elsewhere. Macrophages are not numerous and deposition of pigment is not seen.

The tumor-like mass described in notes on gross appearance shows intense congestion and hemorrhage without evidence of cell destruction, and represents, in all probability, the results of occlusion of blood-vessels at time of operation.

Histologic Diagnosis.—Congestion, increase of reticulum, hyaline degeneration of arteries. (The rather negative histologic appearance is in general that described for splenomegaly with congenital haemolytic jaundice.)

METHODS OF METABOLISM STUDY.—The child was kept in a private room of the University Hospital, with a special metabolism nurse in attendance. The complete metab-

olism study occupied one period of ten days and a supplementary period of five days before splenectomy, and a period of ten days after splenectomy. The first period extended from December 3 to December 14, after which the child went home for the Christmas holidays. While the child was at home a supplementary period of five days for the study of uric acid elimination extended from January 20 to January 24. The return to the hospital was delayed on account of the desire of the attendant physicians to improve, if possible, the blood picture and the general condition. On January 28, two days after re-admission, the spleen was removed, and on February 5, after a lapse of eight days, the post-splenectomy metabolism studies were begun and continued for ten days. On account of the capricious appetite of the child, it was impossible to adhere to a constant dietary, such as the Folin diet, and therefore considerable liberty was allowed. The intake was determined by weighing all foods taken and analyzing portions for nitrogen and fat. This policy was followed in both of the ten-day periods, but not in the supplementary five-day period when the child was at home. Despite the freedom as to diet, the food intake was quite constant in character from day to day, consisting, in the first period, essentially of milk, eggs, cereals, apple sauce, bread, crackers, potatoes, butter, sugar, rice, and tapioca. During the first five days of this period beef, chicken, or fish was allowed once a day; during the second five days these were entirely eliminated, as they were also in the supplementary period of five days before splenectomy and the ten-day period after splenectomy. Thus, except in the first five-day period, the child was on a practically purin- and creatin-free diet. The calorific value of the diet was

adequate. During Periods I and II (Table LV) the subject received approximately 1100 calories a day, or about 60 calories per kilo. of body weight. During Periods IV and V the patient was on a slightly lower calorific intake, but entirely adequate; namely, 960 calories per day, or about 50 calories per kilo. of body weight.

The nitrogen of the food was estimated by the Kjeldahl-Gunning method and the fat by Soxhlet extraction. The urine was collected in twenty-four-hour periods, and portions passed during that period were preserved under toluene in an ice-chest. The urine was acid to litmus at all times.

In the analysis of the urine the total nitrogen was determined by the Kjeldahl-Gunning method; ammonia by Folin's¹²⁰ method; urea by Benedict's^{41a} method; uric acid by Folin's colorimetric method;¹²³ creatin and creatinin by Folin's method;¹²¹ and the hydrogen ion concentration according to Henderson's technique.¹⁷³

In the study of the faeces the fat content was determined by the Folin-Wentworth method;¹²² the iron was estimated by Neumann's method;³¹⁵ nitrogen by the Kjeldahl-Gunning method, and urobilin by a slight modification of the method recommended by Wilbur and Addis.⁴⁷³

Period III (Table LV) was considered a desirable control on account of the high figures for uric acid obtained in the first and second periods. The analyses in this period were therefore limited to those determinations of special interest in this connection.

RESULTS OF METABOLISM STUDY.—In Table LV are presented the results of the study of nitrogen metabolism.

Nitrogen Metabolism.—During Periods I and II, before splenectomy, the subject showed a slightly plus nitro-

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TABLE LV
METABOLISM IN CONGENITAL HÆMOLYTIC ICTERUS BEFORE AND AFTER SPLENECTOMY

Period	Date	Wt. in kg.	Ni- tro- gen in in- take, gm.	Amt., c.c.	Specific gravity	Hydro- gen ion conc.*	Total Nitro- gen, gm.	Urea, gm.	Am- mo- ni- um, gm.	Uric acid, gm.	Total† creat- inin, gm.	Creati- nin, gm.	Faeces; total nitro- gen, gm.	Total nitro- gen output, gm.	Clinical Notes and temper- ature	
I	12/ 4/14	17.7	6.45	480	1.022	6.15	6.09	5.25	0.36	0.468	0.476	0.289	0.217	0.54	6.63	-0.18
	12/ 5/14	...	9.00	530	1.018	5.70	5.46	4.63	0.33	0.468	0.398	0.235	0.154	0.54	6.00	+3.00
	12/ 6/14	...	5.16	480	1.020	5.50	4.99	4.18	0.32	0.468	0.309	0.226	0.096	0.53	-0.37	101.4
	12/ 7/14	...	5.66	400	1.025	5.85	6.35	5.54	0.26	0.516	0.385	0.270	0.133	0.54	6.92	-1.26
	12/ 8/14	...	6.47	550	1.019	6.15	6.54	5.60	0.32	0.490	0.405	0.261	0.167	0.54	7.08	-0.61
	Average	...	6.55	488	21	5.87	5.89	5.02	0.32	0.482	0.389	0.286	0.153	0.54	6.34	+0.12
II	12/ 9/14	...	4.91	280	1.030	6.15	3.96	3.41	0.19	0.410	0.271	0.191	0.093	0.52	4.48	+0.43
	12/10/14	...	6.12	500	1.020	6.30	5.18	4.40	0.31	0.544	0.369	0.289	0.093	0.52	5.70	+0.42
	12/11/14	...	4.12	270	1.028	6.15	3.90	2.75	0.15	0.544	0.211	0.191	0.023	0.52	3.52	+0.60
	12/12/14	...	5.03	320	1.026	6.00	4.07	3.43	0.20	0.460	0.271	0.211	0.035	0.52	4.39	+0.44
	12/13/14	18.2	4.90	360	1.027	6.00	4.98	4.20	0.24	0.788	0.320	0.270	0.058	0.62	5.50	-0.60
	Average	...	5.02	346	26	6.12	4.24	3.64	0.22	0.549	0.288	0.236	0.060	0.52	4.76	+0.26
III	1/20/15	...	4.40	1.019	...	4.59	0.400	0.279	0.225	0.063
	1/21/15	...	4.10	1.029	...	4.29	0.560	0.270	0.269	0.071
	1/22/15	...	4.15	1.033	...	3.51	0.520	0.259	0.207	0.060
	1/23/15	...	6.90	1.019	...	3.81	0.460	0.215	0.215	0.035
	1/24/15	...	5.00	1.024	...	4.31	0.614	0.300	0.270	0.035
	Average	...	4.91	25	...	4.10	0.511	0.271	0.225	0.053

Splenectomy
1/28/15

Splenectomy

IV	$\frac{2}{2}/\frac{6}{7}/\frac{15}{15}$	18.2	7.08	540	1.023	6.00	5.61	4.95	0.22	0.326	0.300	0.203	0.113	0.35	5.96	+1.12	99.6
	$\frac{2}{2}/\frac{8}{9}/\frac{15}{15}$...	6.23	590	1.020	6.00	4.71	4.08	0.21	0.294	0.283	0.180	0.119	0.35	5.06	+1.17	99.0
	$\frac{2}{2}/\frac{9}{15}/\frac{15}{15}$...	6.41	570	1.020	6.00	4.80	4.16	0.19	0.294	0.311	0.197	0.132	0.35	5.15	+1.26	99.8
	$\frac{2}{2}/\frac{10}{15}/\frac{15}{15}$...	4.97	570	1.020	6.70	3.63	3.11	0.12	0.290	0.234	0.176	0.109	0.35	3.98	+0.99	Bronchitis
	$\frac{2}{2}/\frac{11}{15}/\frac{15}{15}$...	4.99	450	1.023	6.80	3.06	2.68	0.10	0.226	0.194	0.176	0.093	0.35	3.41	+1.58	103.2
	$\frac{2}{2}/\frac{12}{15}/\frac{15}{15}$...	6.30	4.36	21	6.30	3.80	0.17	0.286	0.264	0.172	0.107	0.35	4.71	+1.22	102.2	
	Average	...	5.94	544										
V	$\frac{2}{2}/\frac{11}{15}/\frac{15}{15}$...	6.20	630	1.020	5.70	6.15	5.37	0.31	0.296	0.337	0.287	0.058	0.40	6.55	-0.35	99.4
	$\frac{2}{2}/\frac{12}{15}/\frac{15}{15}$...	5.27	350	1.024	5.50	4.83	4.34	0.23	0.272	0.288	0.203	0.041	0.40	5.23	+0.04	98.8
	$\frac{2}{2}/\frac{13}{15}/\frac{15}{15}$...	4.88	530	1.022	6.00	5.64	4.83	0.31	0.320	0.253	0.043	0.40	6.04	-1.21	99.0	
	$\frac{2}{2}/\frac{14}{15}/\frac{15}{15}$...	5.38	360	1.032	6.80	4.08	3.54	0.14	0.224	0.238	0.195	0.050	0.40	4.48	+0.90	Otitis media,
	$\frac{2}{2}/\frac{15}{15}/\frac{15}{15}$	17.5	6.17	430	1.025	6.70	4.73	4.13	0.18	0.218	0.248	0.203	0.052	0.40	5.13	+1.04	99.0
	Average	...	5.57	460	25	6.14	5.09	4.44	0.23	0.266	0.272	0.228	0.049	0.40	5.49	+0.08

* Expressed as negative logarithms.

gen balance. After splenectomy, during Period IV, a great retention of nitrogen occurred, although the intake varied but little from the previous periods and the subject was not gaining in weight. The logical explanation of this change would be either of the following: (1) Reparative processes going on in the body, as indicated by the rapid regeneration of the hæmoglobin and red cells, or (2) the removal of toxic influences, leading to an improvement in the general nutritive condition and thus to the normal nitrogen retention in a healthy child. The utilization of protein was good at all times. The average percentage of total nitrogen eliminated as urea was 85.5 before splenectomy and 87.2 afterwards, figures within the normal range. The differences of the averages may possibly be explained by the changes in one of the other nitrogen constituents of the urine; namely, uric acid.

Uric Acid.—The uric acid output was exceedingly high in Periods I and II. Period I was not purin-free. Period II, however, was practically free from purin intake, notwithstanding which the uric acid output continued on its high level. The urines were highly colored, and when allowed to stand gave a precipitate of uric-acid crystals. In view of this high elimination of uric acid a supplementary study was made (Period III), during which the subject was again placed on a purin-free dietary, consisting mainly of milk, eggs, shredded wheat, and custard. In this period the high output of uric acid, presumably almost entirely endogenous, still persisted. This average (Periods II and III), 0.530 gm. of uric acid, is very close to the highest average of uric-acid output of an adult on a purin-free diet. Few figures for normal uric-acid output in children are to be found in the literature. Closson,⁸⁰

in a child of about seven years of age, found an average output of 0.23 gm. on a purin-free diet.

After splenectomy the average output of uric acid in Periods IV and V decreased 47 per cent. from the average of Periods II and III before splenectomy. It will be remembered that the diets of all these periods were purin-free. The appearance of the urine in these later periods was markedly altered, the dark-red color being replaced by a pale yellow, with never a spontaneous precipitate of uric acid.

Hydrogen Ion Concentration.—On the same general diet the hydrogen ion concentration of the urine remained constant before and after splenectomy. During Period I, on a mixed diet, an average of 5.87 falls within the normal average (5.94) of Henderson and Palmer.¹⁷⁴ On the purin-free diet yielding a more alkaline ash, the hydrogen concentration before operation is in agreement with that after the operation. It is of interest here to note that the hydrogen ion concentration is not appreciably altered by changes in uric-acid content of urine, although, as shown by Blatherwick,⁵⁰ the ability of a urine to dissolve uric acid is a function of the hydrogen ion concentration.

Ammonia Nitrogen.—The ammonia nitrogen in our experiments shows no variations from the normal. Its close agreement with hydrogen ion concentration may be noted; thus a rise in the hydrogen ion concentration is associated with a fall in the ammonia output, and *vice versa*.

Creatinin and Creatin.—The creatinin output showed a great constancy in Periods II and III, before splenectomy, on the purin-free diet. During Period I, on a mixed diet, the output is slightly above these periods. During Period IV, after the operation, the creatinin output fell

to its lowest point, a decrease of 25 per cent. from the average of the other purin-free periods. When the total creatinin (that is, preformed creatinin and creatin considered as creatinin) output of each period is compared, one readily sees that the total creatinin, in all of the periods on creatin-free diet, shows a remarkable constancy. As will be seen from an inspection of the two tables, the decrease of creatinin was accompanied by an increase of the creatin amounting to 91 per cent. of the average. Why, in Period IV, the partition of creatinin and creatin changed without an appreciable change of total creatinin is difficult to state. During the last two days of this period the patient suffered from a bronchitis with a rise in temperature, but that these are explanatory factors hardly seems plausible.

As regards creatin output, with the exception of the first period, which was not that of a creatin-free diet, the output shows a fair degree of regularity. The increased output in Period IV has already been pointed out.

There is a paucity of data on the creatinin and creatin output of children on controlled diets. The results obtained by Folin¹²⁵ on his children offer figures which may serve for comparison.

The great constancy of our total creatinin (including preformed creatinin and creatin as creatinin) output leads us to believe that for the purpose of comparison in children this is the figure to be used rather than the relative or absolute amounts of creatin or creatinin. If we are correct in this view, the total creatinin output agrees very well with other published figures for children, and we therefore believe our creatin and creatinin figures to be within the range of normal variations.

Fats.—The total intake of fats and the separation of fats in the faeces are shown in Table LVI. In this table Periods I and II represent the pre-splenectomy and Periods IV and V the post-splenectomy studies. Each period represents five days.

TABLE LVI

FAT DETERMINATIONS IN A CASE OF CONGENITAL HÆMOLYTIC JAUNDICE BEFORE AND AFTER SPLENECTOMY

Period	Total intake, gm.	Total output, gm.	Per cent. of fat utilized	Total output fatty acids including soaps, gm.	Per cent. fatty acids in total fat output	Total output neutral fats	Per cent. neutral fats in total fat output
Before Splenectomy:							
I.....	222	8.88	96.01	6.8	76.8	2.1	23.2
II.....	223	7.57	96.62	5.1	67.0	2.5	33.0
After Splenectomy:							
IV.....	227	13.56	94.04	10.1	74.4	3.5	25.6
V.....	269	13.55	94.98	9.8	72.3	3.7	27.7

The metabolism of fats shows no abnormal variations. The fat utilization is good, and well within normal limits. As pointed out by Folin and Wentworth,¹²² as total fat increases more of that fat is put out as fatty acids (including soaps).

Iron.—Table LVII presents the results of the exam-

TABLE LVII

IRON ELIMINATION IN FAECES IN A CASE OF CONGENITAL HÆMOLYTIC JAUNDICE BEFORE AND AFTER SPLENECTOMY

Period	Total intake period, mg. (calculated)	Total output period, mg.	Intake per day, mg.	Output per day, mg.
Before Splenectomy				
I	37.69	82.99	3.77	8.29
II				
After Splenectomy				
IV	45.61	41.11	4.56	4.11
V				

ination of the faeces for iron. As the iron in human urine seldom exceeds 0.001 mg., the urine is not included. Analyses for iron were made on duplicate samples of dried faeces representing periods of ten days, before and after splenectomy, respectively.

Thus the first ten days correspond to Periods I and II and the second ten days to Periods IV and V. Periods II, IV, and V represent essentially the same diet. The figures for iron intake were calculated from published records⁴⁰⁶ of iron content of foods, hence no claim is made for the extreme accuracy of those figures. They merely serve to show that the iron content of the diet agreed very closely and would not account for the large difference of output. Those differences in output before and after splenectomy amounted to about 40 per cent. decrease. That the large output of iron in the period before splenectomy is due to the increased elimination of iron consequent on the excessive destruction of red cells seems the most plausible explanation. The decreased elimination after splenectomy, with a close agreement of intake and output, shows a cutting off of this loss and presumably a return to normal elimination.

Urobilin.—Our interest in the urobilin problem has been limited to the influence of the absence of the spleen on the elimination of this substance. In the urine qualitative tests for urobilin gave negative results throughout the experiment. At one time, in a concentrated urine, a faintly positive reaction was obtained with Ehrlich's reagent. The large bulk of this constituent was in the faeces. Because of the small bulk of the child's faeces, and the necessity of utilizing considerable portions for other determinations, the use of the wet faeces for urobilin

determination was impracticable. The faeces were therefore dried in the usual way and placed immediately in well-stoppered bottles. At the end of the experiment the faeces of Periods I and II before splenectomy and IV and V after splenectomy were combined for urobilin estimation. In view of the previous work on this substance, it was to be expected that some of the urobilin would be destroyed, or that most of the urobilinogen would be converted into urobilin, but whether or not this took place we have no means of determining. The fact remains that considerable urobilinogen was still present at the time of analysis. Inasmuch, however, as both sets of faeces were treated alike, this was a more or less constant factor. Five grammes of faeces were extracted with 100 c.c. of acid alcohol and treated as described in method as outlined by Wilbur and Addis.⁴⁷³ The dilution for total mass of faeces was then calculated. The dilution follows:

Dilution required for extinction of urobilinogen and urobilin absorption bands: Periods I and II (combined), 71,250; Periods IV and V (combined), 7954.

These results are in accord with those described by Eppinger,¹⁰⁴ who found, in a variety of clinical conditions accompanied by rapid blood destruction, that the urobilin in the stools sank to normal after splenectomy.

These general results may be summarized as follows:

1. A slight positive nitrogen balance before splenectomy was followed by an increased retention eight days after operation.
2. The output of uric acid showed a decrease of 47 per cent. after operation.
3. In the period directly after operation a change in the partition of creatinin and creatin elimination occurred,

the total creatinin, however, showing but slight change.

4. Other urinary nitrogen constituents showed no variations from the normal, and no change was found in the hydrogen ion concentration.

5. The utilization of nitrogen was good at all times.

6. Fat metabolism was normal.

7. There was a large loss of iron through the faeces before splenectomy, followed by a decided decrease (40 per cent.) after operation.

8. The excretion of urobilinogen and urobilin in the faeces was markedly diminished after splenectomy; the amount after operation was about one-ninth of that excreted before splenectomy.

IN PERNICIOUS ANAEMIA

The second study³⁴² to be presented as a corollary to the above investigation has to do with certain phases of metabolism in an individual with pernicious anaemia characterized by increased haemolysis. The study was limited to the total nitrogen, the uric acid, the iron, and the urobilin and urobilinogen—substances in connection with which changes have been observed in the study of congenital haemolytic icterus. Three periods were studied: one before the transfusion and splenectomy, one two weeks after splenectomy, and the third two weeks later. During each period the patient was on a carefully controlled Folin metabolic diet, and the period was not commenced until the patient had reached an approximate nitrogen balance. The nitrogen of the food and urine was determined by the Kjeldahl-Gunning method, the uric acid according to Folin's permanganate method, the iron by Neumann's method, and the urobilin and urobilinogen according to

the method of Wilbur and Addis. Only negligible amounts of urobilin or urobilinogen were at any time found in the urine.

TABLE LVIII
BLOOD EXAMINATIONS IN A CASE OF PERNICIOUS ANÆMIA BEFORE AND AFTER SPLENECTOMY

Date	Hæmoglobin, per cent.	Erythro- cytes	Leuko- cytes*	Nucleated erythrocytes	Reticu- lated erythro- cytes, per cent.	Remarks
3/28/15	26	1,150,000	4,600	Normoblasts + Megaloblasts +	...	Coagulation time, 4.5 min.
4/ 8/15	25	1,620,000	5,800	Megaloblasts +		
4/15/15	20	1,110,000	2,000	0	4	Hæmolysis in NaCl: partial 0.425, com- plete 0.325
5/ 3/15	20	1,700,000	6,500	0	2	Left hospital for a month
6/ 5/15	28	1,300,000	4,300	Normoblasts +	1	Platelets less than 100,000
6/ 7/15	Transfusion 900 c.c.
6/ 8/15	40	1,810,000	3,800	0		
6/12/15	Splenectomy
6/15/15	37	1,420,000	16,600			
6/21/15	40	2,930,000	12,000	Normoblasts ++		
6/24/15	Severe hæmorrhage from throat
6/24/15	27	After the hæmorr- hage
6/28/15	28	1,640,000	3,700	Normoblasts +		
7/ 9/15	31	1,630,000	6,300	0		
7/15/15	35	2,370,000	6,000	Normoblasts + Megaloblasts +		
7/22/15	48	2,030,000	8,100	Normoblasts +		
7/30/15	55	2,570,000	7,400	Normoblasts +		
8/ 6/15	69	2,300,000	9,100	Normoblasts +	1	Howell-Jolly bodies +
8/16/15	48	3,200,000	8,300	0		
8/24/15	54	3,700,000	8,400	0		
8/29/15	70	3,580,000	9,400	0		
8/30/15	Discharged
1/ 8/16	83	4,400,000	10,500	Normoblast, occasional	...	Count by Dr. S. L. Freeman

* The differential counts of the leukocytes always showed a slight eosinophilia, but were otherwise normal. The erythrocytes showed the changes characteristic of severe anæmia; these became less marked as the anæmia disappeared.

The history and findings in the case will be given briefly. The blood examinations are tabulated in Table LVIII and the metabolic results in Table LIX.

THE SPLEEN AND ANÆMIA

TABLE LIX
EFFECT OF SPLENECTOMY ON ELIMINATION OF URIC ACID, IRON AND UROBILIN IN PERNICIOUS ANÆMIA

Period	Date	Weight in pounds	Nitrogen intake, gm.	Urine			Faeces			Total nitrogen output, gm.	Nitro- gen balance, gm.	Urobilin and urobilinogen
				Amount, c.c.	Total N, gm.	Uric acid, mg.	Total N, gm.	Iron, mg.	Total N, gm.			
I	4/28/15	170 1/4	16.7	1,550	14.8	762	1.46	17	16.26	+0.44	4/9/15 to 4/12/15, 18,300 per day	
	4/29/15	17.6	1,820	16.2	824	1.46	17	17.66	-0.06		
	4/30/15	16.7	1,600	13.7	728	1.46	17	15.16	+1.54		
	5/1/15	172 1/2	17.2	1,680	14.5	788	1.46	17	15.96	+1.24		
	5/2/15	2,200	16.6	2,200	16.2	852	1.46	17	17.66	-1.06		
	Average	16.96	1,770	15.08	791	1.46	17	16.54	+0.42		
II	6/12/15	175	Splenectomy									
	6/24/15	15.7	1,160	10.86	500	1.09	10	11.95	+3.75	6/25/15, 16,500 per day	
	6/25/15	16.8	1,340	12.48	520	1.09	10	13.57	+3.23	6/28/15 to 7/2/15, 16,000 per day	
	6/26/15	17	1,600	14.76	740	1.09	10	15.85	+1.15		
	6/27/15	17.3	1,590	15.54	680	1.09	10	16.63	+0.67		
	Average	16.7	1,420	13.41	610	1.09	10	14.50	+2.20		
III	7/6/15	160 1/4	17.2	1,600	14.2	680	1.97	...	16.17	+1.03		
	7/7/15	16.3	1,380	14.1	680	1.97	...	16.07	+0.23		
	7/8/15	16.6	1,210	13.44	500	1.97	...	15.41			
	7/9/15	17.2	1,210	13.44	620	1.97	...	17.03	-0.23		
	7/10/15	16.8	1,310	15.06	640	1.97	...	16.79	-0.39		
	7/11/15	162	16.4	1,290	14.82	640	1.97	...				
Average	16.75	1,360	14.32	624	1.97	...	16.29	+0.46			
	8/22/15	190	8/18/15 to 8/22/15, 2,300 per day	

Clinical Notes.—The patient, a man, aged forty, had complained for two years of weakness, dizziness, dyspnœa, and oedema. These symptoms were steadily becoming worse. In other respects his history is unimportant. The physical examination revealed nothing noteworthy other than the signs of intense anaemia, associated with a lemon-yellow pallor. The liver-edge was just palpable. At operation the spleen was found to be about three times its normal size, weighing 340 gms. The pathologic examination of the spleen showed chronic diffuse and follicular hyperplastic splenitis, with passive congestion and excessive pigmentation. The Wassermann was negative. On account of a constant eosinophilia, repeated careful examinations were made of the stools for ova or parasites, but with negative results. The other laboratory reports are unimportant. The patient improved gradually after the splenectomy, and six months later was doing fairly arduous work, apparently in perfect health.

The figures as given in Table LIX show that but little change in the elimination of uric acid and iron took place as a result of the splenectomy. The direction of the changes is, however, in each instance, in accord with the more pronounced changes in the study of congenital haemolytic jaundice, in which the haemolytic factor is more marked.

In view of the fact that the nitrogen balance is practically identical in the first and third periods, it may be concluded that splenectomy in this case, as in other cases reported in the literature, has no permanent effect on the total nitrogen balance. The distinct positive balance during the second period is of interest, but probably of no significance in relation to splenic function. The uric-

acid elimination before operation cannot be said to be other than a high normal figure, and the lower post-operative figures are still within normal range; but when it is considered that the diet and régime in general were identical before and after operation, the lowered output after operation is definite and significant. The same can be said of the figures for the iron elimination.

In the combined urobilin and urobilinogen elimination a definite change is noted following the splenectomy. Two weeks after splenectomy the diminution in the urobilin output was negligible, the difference between 18,300 units per day and 16,000 being too slight to permit of significance being attached to it. Two months after splenectomy, however, at a time when the blood count showed a pronounced and most satisfactory improvement, the urobilin output had fallen to one-seventh of its former figure and had reached a low normal elimination.

These observations may be summarized briefly as follows:

1. A slight positive nitrogen balance before splenectomy was followed by an increased nitrogen retention fourteen days after operation and a return to the pre-operative balance after one month.

2. The output of uric acid, although never exceeding normal limits, showed a decrease of 22 per cent. after operation.

3. The output of iron through the faeces, although never above normal, showed a decrease of 40 per cent. after operation.

4. The excretion of urobilinogen and urobilin in the faeces before splenectomy was about three times the normal; two months after operation the output was about one-seventh of that before splenectomy.

DISCUSSION

The literature concerning the relation of the spleen to metabolism may be considered under five heads: (1) Studies both before and after splenectomy for disease of the spleen in man; (2) studies in man after splenectomy; (3) studies of congenital haemolytic jaundice; (4) studies of anaemia; (5) studies of the effect of removal of the normal spleen.

1. In only three instances other than those reported above have metabolic studies been made both before and after splenectomy for diseases of the spleen in man. Two of these are Umber's studies of Banti's disease, and the third Minot's study of pernicious anaemia. Umber studied two individuals splenectomized for Banti's disease, and Minot one in whom the spleen was removed as a last resort in pernicious anaemia. One of Umber's subjects⁴⁴⁴ was a boy of fifteen with anaemia and icterus. The post-operative period of study covered twelve days and began twenty-four days after the operation. The diet was purin-free, and a fully-controlled metabolic study was made. The results showed no pronounced variation in the distribution of the urinary constituents which could be attributed to the absence of the spleen. Umber makes a point, however, of the fact that after removal of the spleen it was easier to obtain nitrogen equilibrium, and attributed the pre-operative pathologic destruction of protein to a toxic cause. His figures show also a somewhat greater output of purins before the operation than after. In another case of Banti's disease described in this report⁴⁴⁴ the "toxic" disturbance of metabolism was not present and splenectomy was not done. In a later study⁴⁴⁴ Umber describes a young man of twenty-one suffering from what

he considers as the "toxic" type of Banti's disease. Splenectomy led to striking improvement. The metabolism study of this case was limited to a comparison of total nitrogen intake and output before and after splenectomy. The results confirm his former observation, namely, that a persistent negative balance before splenectomy changes to a positive balance after splenectomy. The post-splenectomy study was made three months after operation. Minot's²⁹³ patient was a colored woman, aged thirty-five, on whom the second period of metabolic studies were begun fifteen days after splenectomy and blood transfusion. The figures given for five twenty-four-hour periods before and six after splenectomy are not for consecutive days. The examination included total nitrogen in urine and faeces, and urea and ammonia in the urine. The chief results were a change from a slight negative to a slight positive nitrogen balance and an increase in percentage of urea after splenectomy. The uncertainty of the food intake in the period before splenectomy, the low caloric intake, and the shortness of consecutive periods of observation make these balances of doubtful value.

2. The following studies made after splenectomy have no fore-period for comparison. Lo Monaco²⁵³ found in a splenectomized individual no important change in uric-acid elimination.

Mendel and Gibson,²⁸⁴ in the case of a man with enlarged spleen and secondary anaemia following malaria, studied the metabolism (total nitrogen, urea, uric acid, ammonia, phosphorus, chlorides, and sulphates) after splenectomy, but found no striking variation from the normal distribution of the urinary components.

Likewise, Moraczewski,²⁹⁷ who made some studies of

both nitrogenous and mineral metabolism in a man of fifty-one, seven months after splenectomy for "spleen tumor" (malarial), found no important variations. His observations, however, were few in number and were made in the course of an attack of pneumonia which rendered matters of diet and control difficult.

3. The only carefully-conducted and complete study of the metabolism in congenital haemolytic icterus is, so far as we are aware, that of McKelvy and Rosenbloom.²⁶² The patient, a girl aged eleven, on a Folin diet, was studied for six days. The total nitrogen, fat, and mineral constituents of the food were determined and both urine and faeces studied as to nitrogenous and mineral constituents and the faeces as to fat. During a period of six days there was a loss of 4.06 gms. of nitrogen, which the authors suggest may be due to a toxogenic destruction of protein. The nitrogen partition of the urine was normal except in the case of the uric-acid nitrogen, which was increased. This increase, the writers state, might be due to the increased liberation of nucleoproteins through haemolysis of the erythrocytes. The study of mineral metabolism showed a loss of sulphur, iron, calcium, and magnesium, and a retention of phosphorus. The fat metabolism was normal. No metabolism studies were made after splenectomy.

In a woman, aged thirty-nine, with "chronic family jaundice," Tileston and Griffen⁴³⁵ studied, for three successive days and an added odd day, the output of ammonia, urea, creatin, and creatinin on a purin-free and creatin-free diet. They found the elimination of creatinin and urea to be essentially normal, ammonia somewhat high, and uric acid distinctly increased. However, it should be

noted that only one determination of uric acid was made.

Haal,¹⁶¹ in a case of family haemolytic jaundice, found an increased excretion of uric acid and of iron.

4. As the changes in metabolism in various types of anæmia have recently been summarized by Minot, we will not present this literature in detail. The opposing views are represented by Rosenqvist and von Noorden. Rosenqvist,³⁸⁴ in pernicious anæmia and bothriocephalus anæmia, found variations in nitrogen elimination, with periods of alternate increased and decreased excretion. In bothriocephalus anæmia a well-marked loss of nitrogen, while the worm was in the body, was followed, after removal of the worm, by a nitrogen retention. Rosenqvist concluded that in both types of anæmia a pathologic decomposition of protein is present. Von Noorden³¹⁸ opposes this view, and as a result of his studies concludes that protein decomposition is not increased as the result of anæmia of the ordinary type. The variations in output, he believes, may be explained by the alimentary and renal disturbances which accompany anæmia. That an increased output of nitrogen may occur in anæmia due to parasites is admitted, as is also the possibility in non-parasitic anæmias of a temporary increase in the output of nitrogen as the result of a sudden destruction of large masses of red cells.

As to uric-acid output, von Noorden refers to Rosenqvist's high figures and to other observations and concludes that, as a rule, in anæmia the output is normal, but sometimes rises, as in Rosenqvist's work, to twice the normal amount.

It is noteworthy that in bothriocephalus anæmia Rosenqvist found that after removal of the parasite the purin output increased temporarily and then returned to normal. This temporary increase he explains as due to the regen-

eration and increased metabolic activity of the blood and somatic cells consequent on the removal of the toxic agent. As the cells recovered their normal equilibrium the output of the purins fell to normal level.

Halpern,¹⁶³ studying one case of pernicious anaemia and one of splenic anaemia, found normal values for the various urinary constituents. His figures for purin output are in no way abnormal.

Samuely,³⁹¹ in his studies of metabolism in dogs rendered anaemic by poisoning with pyrodin, found no essential changes in protein metabolism.

5. The literature of splenectomy in man for conditions other than chronic anaemia, as, for example, gunshot wound, rupture, cyst, etc., shows that no metabolism studies have been made in such conditions. Conclusions concerning the effect on metabolism of removal of the normal spleens in the normal individual must therefore be based on observations on animals.

Metabolic studies before and after splenectomy in animals, as we have shown elsewhere (see p. 181), indicate that the removal of the spleen does not influence protein metabolism.

As to metabolism studies of substances other than protein and its derivatives, the same paucity of data exists.

The literature contains no records of the examination of the faeces for fat before and after splenectomy. Tilles-ton and Griffen, in one of their cases of chronic family jaundice, studied, without result, the fats of a single stool. McKelvy and Rosenbloom, in their case of congenital haemolytic jaundice, report normal fat metabolism.

The literature of iron metabolism is, at best, unsatisfactory, and this is especially true of work on the relation

of the spleen to iron metabolism. Most of the work is based on Schmidt's³⁹⁵ conclusions, drawn from the results of the feeding of iron-poor food to normal mice, that the organism possesses great power of conserving the iron and of reutilizing it through some form of intermediary metabolism. In this connection Schmidt regards the liver as the depot for iron from the food, and the spleen as the depot for iron from tissue and erythrocyte catabolism.

The experimental evidence concerning the relation of the spleen to iron metabolism, which we have described in detail elsewhere (see p. 112), is contradictory. Asher and his associates, Grossenbacher¹⁶ and Zimmermann,¹⁹ claim that the dog after splenectomy eliminates an increased amount of iron. Our observations do not support these findings. In our early work we occasionally found a slight increase in the faeces one and two weeks after splenectomy, but in later studies an increase was never found except once in an anaemic animal. We are therefore inclined to view a disturbance of iron elimination in the dog as due to an associated anaemia rather than to the disturbance of some splenic function.

No observations in man, other than our own (see Table LX) on iron elimination both before and after splenectomy, are at hand. Bayer,³⁹ in the study of iron elimination after splenectomy for (1) rupture of the spleen and (2) Banti's disease, compared his results with those obtained in normal individuals. He found an increased output soon after splenectomy in the case of spleen rupture, but later the elimination returned to normal; in the case of Banti's disease the elimination did not differ from the normal controls. A similar study has been made by Roth³⁸⁵ with like results: iron elimination was studied

in (1) a man, twenty-six years of age, whose spleen had been removed three years before because of splenomegaly associated with a type of haemolytic jaundice, presumably congenital, and (2) a man, thirty-seven years old, whose spleen had been extirpated one month before for rupture due to trauma. On both low and high iron diets the amount of iron absorbed by each, in relation to an estimated intake, was practically the same, but the output in the second individual was twice that of the first; thus on the same iron intake the former eliminated 6.25 mgm. and the latter 12.18 mgm. per day. These figures are in accord with the extreme for normal individuals, but one cannot avoid a query as to whether the time elapsed since splenectomy, three years as compared to one month, is not a factor in the widely different figures, or, again, whether the earlier anaemia in the first case may have been a factor. Indeed, the paucity of iron figures and the wide variation in figures for normal individuals render their interpretation exceedingly difficult. This may be seen in Table LX, in which we have grouped all analyses, for normal and anaemic individuals, which we have been able to collect from the literature. McKelvy and Rosenbloom's studies of congenital haemolytic jaundice before splenectomy show, as do ours, that the elimination of iron in this disease is, on the basis of normal figures in the literature, increased. This increase they explain as due to the great destruction of red cells.

From a review of the literature it is evident that in anaemia, with or without splenic disease, the majority of investigators have experienced difficulty in obtaining a nitrogen balance. Umber,⁴⁴⁴ in his study of Banti's disease; Minot,²⁹³ in pernicious anaemia; McKelvy and Rosen-

bloom,²⁶² in congenital haemolytic icterus, and Rosenqvist,³⁸⁴ in pernicious anaemia and bothriocephalus anaemia, all report a pathologic destruction of protein. Umber⁴⁴⁴ goes so far as to urge this "toxic destruction" as a criterion for operation.* Von Noorden³¹⁸ alone opposes this theory of increased destruction of protein in anaemia. In the two cases we report, no difficulty was experienced in obtaining a positive nitrogen balance before operation: feeding was not forced, the patients merely satisfying their natural desires for food. Nevertheless, the increased retention immediately after the operation on the same nitrogen intake would appear to support the theory that some toxigenic influence had been removed. To this influence, however, must be added as a cause of retention the higher level of reparative processes going on in the body, as, for example, in the bone-marrow and possibly other organs. It is, however, difficult to reconcile our slight positive balance with Umber's marked negative balance, before operation.

As regards the elimination of uric acid in anaemia, with or without disease of the spleen, one finds the general view to be that the elimination is high. Rosenqvist (in 1903), as a result of his studies of pernicious anaemia and bothriocephalus anaemia, reports large outputs of uric acid, sometimes twice the normal amount. He finds that after the removal of the worm in the latter condition there is first an increased elimination of purins and then a return to the normal. His explanation for this is an increased metabolic activity of the blood and somatic cells following the removal of the toxic agent. In our case, after removal

* Luce,²⁵⁶ Müller,³⁰⁷ Lommel,²⁵² Grosser and Schaub,¹⁵⁶ have failed to find an increased destruction of protein in Banti's disease.

of the spleen, we found the reverse condition—an immediate drop to normal. That in our first case an increase took place between the time of operation and the beginning of our first post-splenectomy period (eight days after operation) we cannot say. When, however, one inspects the results of blood examination after operation, it is observed that the increase in red cells and haemoglobin was steady and gradual, no greater during the first eight days after operation than during the subsequent ten days of our metabolism period, so that regenerative processes were at best gradual.

Umber, in his studies, does not report uric-acid output, but groups his findings under total purins, of which he found, in Banti's disease, a somewhat greater output before operation than after. Haal, in family haemolytic jaundice, found an increased excretion of uric acid. Von Noorden gives as his opinion, based on a review of the literature, that in anaemia the output may be normal, but is sometimes increased. Lo Monaco, and Mendel and Gibson report no change in uric-acid excretion after splenectomy, but present no pre-splenectomy studies for comparison.

In congenital haemolytic icterus McKelvy and Rosenblum report higher uric-acid output, but present no studies after splenectomy. They give as an explanation for the increased output the greater formation of nucleoprotein resulting from the destruction of red cells. Their explanation seems to be inadequate, for it is difficult to imagine the destruction of red cells as being the sole source of this large output of purin. It may, to some extent, be a factor, but the toxic influence on the somatic cells generally of bile products would appear to be a factor of greater importance. The sallow discoloration of the skin

in the disease is indicative of the general dissemination of a substance absorbed directly or indirectly from the bile, and our knowledge of the toxic influence of bile constituents offers a possible explanation for a widespread state of cell degeneration and consequent repair which would account for increased output of products of nuclein metabolism.

The only experimental studies which seem to have any bearing on this problem of protein metabolism are those of Jackson and Pearce,³³⁸ who used haemolytic immune serum to produce liver necrosis and made detailed studies of associated changes in metabolism. The changes caused by haemolytic serum—anaemia, jaundice, and cell degeneration—represent as close an approach, aside from chronicity to conditions in congenital haemolytic jaundice as can be brought about experimentally. It is of interest that under such experimental conditions an increased output of total nitrogen, rest nitrogen, purins, and phosphorus was observed. These changes were explained as due to cell autolysis consequent upon the necrosis of liver tissue; when the necrosis was absent, little metabolic change was evident. It is impossible to make an exact parallel between an acute experimental lesion in animals and a chronic disease in man, especially as the pathology of congenital haemolytic jaundice offers no evidence of focal liver necrosis, but in view of the attempts of the several investigators we have quoted to show a toxic destruction of tissue in haemolytic anaemias, the experimental studies of Jackson and Pearce are suggestive.

The improvement in functional equilibrium after splenectomy, shown by the studies of protein metabolism, are emphasized by the studies of the exchange of iron. Our

findings in congenital haemolytic jaundice and pernicious anaemia can be explained, when compared with the direct blood examination, only on the basis of an increased blood destruction before splenectomy and the removal of a haemolytic factor by the operation. And this view is tenable despite the fact that the figures for iron elimination before splenectomy are well within those given for normal individuals (see Table LX). A study of figures for intake and output of iron and of elimination before and after operation leaves no doubt as to the influence on the iron exchange. Our results cannot be brought into relation with other investigations, as in no studies before ours has iron elimination been studied both before and after splenectomy.

The results of our study of urobilin are in accord with the older views as to the source of this substance (that is, excessive blood destruction), and also with the views of Eppinger concerning the decrease of urobilin after splenectomy in various diseases of the blood. The study of urobilin elimination has become a matter of considerable importance in prognosis after splenectomy. Its increased elimination is usually associated with other evidences of increased haemolysis, as high excretion of iron and uric acid and a discoloration of the skin, and its decrease after splenectomy is considered as indicative of the checking of haemolysis and as justifying a favorable prognosis. This is particularly true of congenital haemolytic jaundice, but observations concerning urobilin elimination in pernicious anaemia are somewhat contradictory. Thus Robertson³⁷⁸ emphasizes the fact that cases which had shown a high urobilin excretion before splenectomy and in which, after splenectomy, the urobilin output exhibited only a transient reduction, or none at all, did not show as much improve-

TABLE LX
ELIMINATION OF IRON IN HEALTHY AND ANÆMIC INDIVIDUALS

Observer	Sex	Age	Iron in mgm.		Remarks
			Intake per day	Output per day	
Lehmann, Muel- ler, Munk, Zuntz ²⁴⁴	Male	26	Fasting	7.3 * ⁵	Professional fasters; 10 and 6 day periods re- spectively
	Male	21	Fasting	7.7	
Stockman and Greig ⁴¹⁸	Male I	20	6.2* ¹ 5.6	6.32* ⁴ 11.46* ²	Healthy individuals
	Male II	35	6.2	8.33	
Von Wendt ⁴⁶⁶	Female	23	3.5	3.73	Nine periods of observa- tion on two healthy in- dividuals
	Male 1	..	11.0 * ¹	9.0* ⁴	
Sherman ⁴⁰⁷	..	2	6.0	11.0	
	..	3	10.0	14.0	
	..	4	8.0	9.0	
	..	5	17.0	42.0	
	..	6	7.0	15.0	
	..	7	19.0	24.0	
	..	8	28.0	34.0	
	..	9	27.0	32.0	
	Male	..	5.7 * ³	5.5 * ⁴	Three healthy individuals
McKelvy and Rosenbloom ²⁶²	Male	..	6.5	8.7	
	Male	..	7.1	12.6	
Roth ³⁸⁵	Female	11	8.8 * ¹	32.51* ⁴	Congenital haemolytic jaundice—5 day period
	Male	26	90.0 * ³ 150.0	6.25* ⁴ 4.32	Hæmolytic anæmia. Sple- nectomized 3 years pre- viously
Bayer ³⁹	Male	37	90.0 200.0	12.18 33.07	Splenectomized one month previously for trauma of spleen
	Male	16	240.0 * ¹ 140.0 130.0 80.0 300.0	9.38* ⁵ 7.41 14.54 5.92 26.73	Two weeks after splenec- tomy for traumatic spleen rupture. Three months later
Bayer ³⁹	Male	16	240.0 140.0	8.40 7.29	Control: Fracture of tibia
	Male	16	130.0 80.0 300.0	8.57 3.57 23.49	Control: Osteomyelitis; operation 14 days be- fore
Bayer ³⁹	Female	19	130.0	13.86	Morbus Banti; 2½ years after splenectomy
	Female	25	130.0	10.20	Morbus Banti; ½ year after splenectomy
Bayer ³⁹	Female	27	60.0	21.46	Morbus Basedow; before thymectomy
			60.0	32.70	Three weeks after

TABLE LX—*Continued*
ELIMINATION OF IRON IN HEALTHY AND ANÆMIC INDIVIDUALS

Observer	Sex	Age	Iron in mgm.		Remarks
			Intake per day	Output per day	
Goldschmidt, Pepper and Pearce ¹⁶⁰	Male	22	60.0	12.83	Six weeks after
			60.0	19.00	Ten weeks after
	Male	5	130.0	3.59	Morbus Banti; before splenectomy
Pepper and Aus- tin ³⁴²	Male	40	3.77 ^{*3}	8.29 ^{*5}	Congenital haemolytic jaundice. Before sple- nectomy. 10 day period
			4.56	4.11	After splenectomy, 10 day period
			16.5 * ³	17.0 * ⁵	Pernicious anaemia. Be- fore splenectomy, 5 day period
			16.5	10.0	Two weeks after splenec- tomy, 4 day period

*¹ Iron intake determined by actual analysis.

*² Two periods on same individual; bulk of faeces in second period twice as great as in first.

*³ Iron intake estimated from tables.

*⁴ Urine and faeces.

*⁵ Faeces only.

ment in other respects as did those cases in which the urobilin output was permanently reduced. On the other hand, Lee, Vincent, and Robertson²⁴¹ state that in some cases of severe anaemia which showed marked symptomatic improvement for several months after splenectomy there was in the post-operative period a return to a continued high excretion of urobilin.

More work is necessary before this problem can be considered as settled, and it is to be hoped that metabolism studies before and after splenectomy will include not only the anaemias, but studies in essentially normal individuals, such as those with simple lesions of the spleen, unaccompanied by anaemia. Studies of this latter type would eliminate present doubt as to the importance of the factor dependent on the absence of the function of the normal

spleen and thus offer the essential control, now lacking, for the correct interpretation of the metabolic disturbances; that is, whether they are due to anæmia or the diseased spleen, or both.

For the present, however, it seems justifiable to conclude that splenectomy in the haemolytic anæmias is, as a rule, followed by a reduction in the elimination of uric acid, iron, and urobilin, changes indicative of decreased destruction of tissue and blood elements.

Part II.

CLINICAL OBSERVATIONS BY

E. B. KRUMBHAAR

CHAPTER XI

CLASSIFICATION AND ANALYSIS OF TYPES OF SPLENO-MEGALY ACCOMPANIED BY ANÆMIA.

ENLARGEMENT of the spleen is a common accompaniment of many clinical conditions, but in this chapter will be considered only such splenomegalies as are accompanied by anæmia; as, for example, Banti's disease, splenic anæmia, Gaucher's disease, hæmolytic jaundice, and pernicious anæmia, conditions in which the enlarged spleen seems to have an important relation to the anæmia.

The connection between chronic enlargement of the spleen and marked anæmia without leukocytosis, as denoted in the term "splenic anæmia," was first established fifty years ago by Gretzel,¹⁵⁴ in Griesinger's clinic, in Berlin. He describes the case of a child, ten months old, suffering from dysentery and severe anæmia, with considerable enlargement of the spleen and a lesser degree of enlargement of the liver and lymph-nodes. Examination of the blood, by the crude methods then in vogue, showed that the proportion of white to red cells was not increased. Although later authorities have considered this to have been either a case of Hodgkin's or of von Jaksch's disease, it unquestionably served to differentiate a new clinical condition; that is, it was made clear that the disease in question was not leukæmia. Five years later H. C. Wood¹⁵⁵ described a "splenic variety" of pseudoleukæmia, characterized by greatly enlarged spleen and severe anæmia, but without leucocytosis. Other case reports of like nature appeared from time to time, but it was not

until 1900 that Osler's³²² work familiarized the English-speaking public with the condition now generally known as splenic anaemia. The earlier descriptions, made at a time when the pathological anatomy of the spleen was little understood and when the methods of examining the condition of the blood were very inexact, constituted a distinct advance, in that they differentiated a new type of disease previously confounded with leukæmia. The term "splenic anaemia" is now known, however, to include several distinct types, and its use should be restricted, if not, indeed, discarded entirely. The fact that in most cases the etiology or pathogenesis of this group of diseases is not yet clearly understood is no more an argument for continuing to group them under such loose terms as "splenic anaemia" or "splenomegaly with anaemia" than it would have been to continue to group typhoid fever with typhus fever until the discovery of the bacillus typhosus. Though much still remains to be learned in regard to them, and though probably even their present eponymic and cumbersome names are only temporary, and will be found to include one or more entities, nevertheless it has already become more profitable to deal with them as independent affections. The inconvenience resulting from the present use of the term "splenic anaemia" is quickly demonstrated to anyone making a critical survey of the literature. Statistical summaries, including valuable detailed information, are thus frequently rendered useless when, on analysis, they are found to include several independent types of splenic disease. Cases are occasionally reported under such headings as "simple hypertrophy of the spleen" or "idiopathic splenomegaly," in which not only has anaemia been absent, but histological examination of

the spleen has failed to reveal any peculiar pathological change (Kidd,²¹² Senator and Krause⁴⁰⁴). These reports, however, are so few in number and are based on such slight evidence that the conditions described are not as yet entitled to an independent consideration. In most cases names such as those quoted are used for the want of a more accurate designation, a practice that should be discouraged.

Certain other diseases, in which the spleen is involved or said to be involved, will not be discussed in this chapter. These are (1) various hæmolytic anaemias of specific origin (dibothrioccephalus latus and uncinariasis), (2) lesions of the spleen associated with thrombosis of the portal vein or artery, and (3) some splenic types of cirrhosis of the liver. It must suffice thereby to indicate such relations. Also, all types of enlarged spleen in which certain features render differentiation easy (as leukæmia, pseudoleukæmia, and changes secondary to obstruction or infection, as in heart-disease, typhoid, kala-azar, syphilis, etc.) will be omitted from consideration. This leaves the more definitely differentiated offsprings of "splenic anaemia" as Gaucher's disease (or large-celled splenomegaly), Banti's disease, v. Jaksch's pseudoleukæmia infantum, the Hayem-Widal or acquired form of hæmolytic jaundice with splenomegaly, and the Chauffard-Minowski or congenital or familial form of the same. Some of the differential points of these diseases are indicated in Table LXI.

GAUCHER'S DISEASE

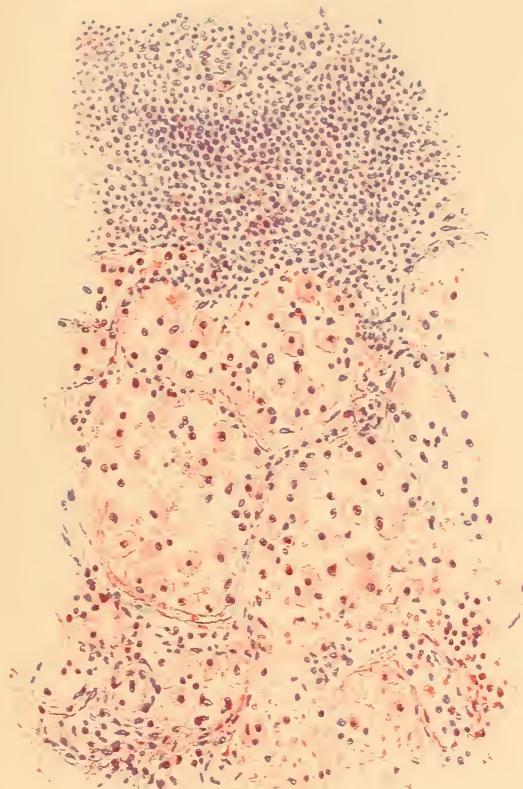
Gaucher's disease, or large-celled splenomegaly, was probably the first variety to be differentiated on account of its peculiar anatomical picture. Although in reality possessing little in common with the other forms of splenic

THE SPLEEN AND ANÆMIA

TABLE LXI
THE SPLEEN AND ANÆMIA. DIFFERENTIAL DIAGNOSIS

Etiology	Pathology of spleen	Family history	Time of onset	Duration	Icterus	Splenomegaly	Hemorrhages	Anæmia	Resistance of red blood cells	Retinolated red blood cells	Liver	Treatment
Gaucher's disease	Toxin? Infection? Tumor?	Peculiar cell hyperplasia	Occasionally positive	Childhood	Many years	Rate	++	Slight occasionally	?	?	-	+
Banti's disease	Toxin? Infection?	Hyperplasia fibrosis	Negative	Adult life	Few years	Rare	+	Slight to severe occasionally	Normal	+	First + Later -	Splenectomy
v.Jakob's disease	Rachitis?	Hyperplasia fibrosis	Negative	Infancy	Several months	Rare	++	?	?	+	+	Sp. Iron, arsenic etc.
Hayem-Widal	1. Primary? 2. Secondary to infection	Congenital a. n. d. pigmentation	Negative	Any age	Many years	Present	++	Rare	Severe	-	+ or normal	Sp. Iron, arsenic etc.
Chaufard-Minkowski	Heredity	Congenital a. n. d. pigmentation	Positive	Congenital or childhood	Many years	Present	++	0	Diminished	+	-	Sp. Iron, arsenic etc.
Pernicious anæmia	Enterogenous toxin?	Fibrosis	Negative	Adult life	Few years with remissions	Very rare	Rare	Severe	Increased	+	- or +	Normal Iron, arsenic, etc. (splenectomy questionable)

PLATE II



Histology of Gaucher's disease. Alveolar arrangement of large vesicular cells with small concentric nuclei.

anaemia, it is here considered in some detail, on account of the similarity of the clinical picture and from the fact that it is still included by many writers under that heading.

PATHOLOGY.—First described by Gaucher,¹³⁹ in 1882, as a primitive epithelioma, it was later shown not to possess most of the characteristics of malignancy. Bovaird⁵⁴ called it a simple endothelial hyperplasia, and Brill and Mandlebaum⁵⁷ showed that the cells developed simultaneously from the endothelium of the spleen, lymph-nodes, and bone-marrow. Diagnosis during life is not easy, but the characteristic large vesicular cells, with small eccentric nuclei, which block the sinuses of the spleen and lymph-nodes or are crowded about the liver lobules, render the recognition of the condition a simple matter to the pathologist. A careful analysis by Brill and Mandlebaum has reduced the number of authentic reported cases to fourteen. Since that time cases have been reported by Herrmann,¹⁷⁵ Knox, Wahl and Schmeisser,²¹⁹ and an unpublished case studied by Veeder,⁴⁵¹ in all of which the characteristic vesicular cells were found in the spleen and elsewhere. Mandlebaum and Downey,²⁷³ however, reject Knox, Wahl, and Schmeisser's two cases, and restrict true Gaucher's disease to those cases in which the characteristic cells are arranged either in enormously enlarged sinuses surrounded by thick connective-tissue walls, or as solid masses surrounded by connective tissue. They consider that cases in which parenchymal cells (as in liver and adrenal) have undergone lipoid (?) change should not be considered as true Gaucher's disease. Knox, Wahl, and Schmeisser, on the other hand, hold that "any disease in which the spleen, together with any other organ, shows numerous large, pale granules or finely vacuolated cells

giving the characteristic microchemical reactions for lipoids and showing a tendency to be widely distributed, belongs to this (Gaucher) group."

An acute form of Gaucher's disease, with fatal termination after fifteen months, has been described by Niemann,³¹⁶ under the title of "an unknown disease picture." The finding of characteristic large, endothelial-like vesicular cells at autopsy makes it probable that an acute form of this disease must also be recognized.

ETIOLOGY.—Its etiology is still in doubt. Malignancy, having been discredited, various theories of endogenous toxins, splenic enzymes, or of infection have been offered, but none is supported by conclusive evidence. It is claimed that a similar histological picture can be produced in animals by the forced feeding of cholesterol (McMeans,²⁶³ Luden²⁵⁷).

SYMPTOMATOLOGY.—The excellent summary of Brill and Mandlebaum reveals the following clinical picture: The disease begins insidiously in infancy or childhood (usually before the thirteenth year) and pursues a very chronic course (average of twenty years). A history of similar trouble in the family is frequently elicited. No great disturbance in the health of the individual occurs until the disease has persisted for some time, when distinct anæmia appears and, as in Banti's disease, a definite tendency to submucous or subcuticular hemorrhages. These, however, are never fatal, and death usually occurs from an intercurrent affection. The most prominent symptom is the progressive enlargement of the spleen, which may reach greater proportions than in any other disease, eventually filling most of the abdomen. The abdominal discomfort produced by the enlarged spleen may be the first

indication of the disease. As in Banti's disease, the blood changes are not very characteristic. The anaemia of chlorotic type is never very severe (average red blood-cell count of advanced cases being 3,700,000). A definite leukopenia is usually found, though the differential count remains unchanged. No enlargement of the superficial lymph-nodes can be found, and jaundice and ascites are rare.

Liver enlargement, secondary to that of the spleen, may eventually reach considerable proportions. A brownish discoloration of the skin has been noticed with a "peculiar yellowish, wedge-shaped thickening of the conjunctivæ, commonly seen on both sides of the corneæ." In spite of the rather negative character of the symptoms, the disease has been recognized during life in at least four of the seventeen authentic cases, with a confirmation of the diagnosis by histological examination of material obtained by splenic puncture or after splenectomy.

PROGNOSIS.—According to Herrmann, Roth, and Bernstein,¹⁷⁶ splenectomy has been tried in nine cases, with three deaths.* This probably represents too high a mortality, but, on the other hand, an ultimate cure can hardly be expected when the disease is known to exist independently in the bone-marrow, lymph-nodes and elsewhere. Little is known concerning the power of the bone-marrow to react. The resistance of the red cells and number of skeined cells (as evidence of power of blood regeneration) have not been studied, but, in view of the absence of signs of increased haemolysis, one would not expect to find any noteworthy changes by such examinations. On the other

* Veeder's unpublished case, with recovery after splenectomy, increases these figures to ten cases with three deaths.

hand, the red bone-marrow, usually present at autopsy, would point to efforts at regeneration.

To sum up, this disease, despite the fact that it is frequently included among the "splenic anæmias," has but little in common with them clinically beyond the chronically enlarged spleen and anæmia. Pathological examination should always allow the proper diagnosis to be made.

BANTI'S DISEASE

SYMPTOMATOLOGY.—Banti's disease, or splenomegaly with hepatic cirrhosis, was first described by Guido Banti³⁰ in 1894, and the clinical picture presented at that time still holds, although the etiology and pathogenesis of the disease remain in almost as great obscurity as at the time of its differentiation. Usually occurring in young, otherwise healthy adults and running a chronic course, its symptomatology may be divided into three periods. In the first or pre-ascitic period, usually lasting several years, a gradually increasing weakness and pallor is noticed, with digestive disturbances and abdominal pain, which may first call attention to the enlarged, smooth, hard spleen. A tendency to hemorrhages with a moderate anæmia of chlorotic type is usually present, but may be postponed until the later stages. There is nothing specially characteristic of the anæmia, the increase of urobilin being the most significant sign of increased blood destruction. The resistance of the red cells is unchanged; signs of a regenerating bone-marrow, as nucleated and reticulated red cells, are slight or absent. After splenectomy, however, an increased resistance of the cells may be noted, and may be marked.*

* In a case I recently examined after splenectomy complete haemolysis did not occur in salt solution as low as 0.25 per cent. No nucleated red cells were found, but reticulated forms were more numerous than before splenectomy.

PLATE III



Spleen of Banti's disease. (From service of Dr. Le Conte, Pennsylvania Hospital.)

A slight or moderate amount of leukopenia is characteristic.

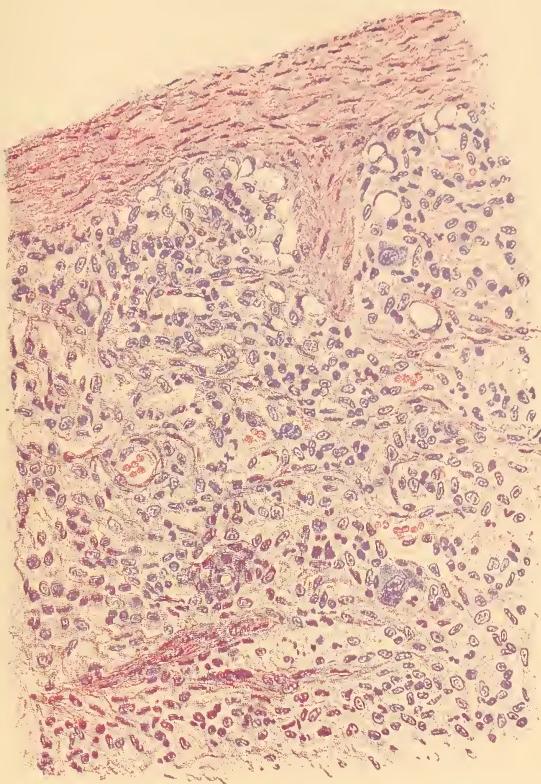
The second, or intermediate, stage lasts but a few months, and is characterized by scanty, high-colored urine containing an excess of urobilin, by attacks of dyspepsia and diarrhoea, and by slight increase in the size of the liver.

The third stage is ushered in by the symptoms of cirrhosis, a recurrent, painless ascites, occasionally slight jaundice, shrunken liver, and increasing anaemia and emaciation. After a few years an intercurrent infection or fatal hemorrhage is the terminal event. It is hardly necessary to say that such a picture is subject to variation, and that in some cases the three periods cannot be distinguished. The first and second of these periods are usually considered as "splenic anaemia."

ETIOLOGY AND PATHOGENESIS.—In spite of the great amount of work done on Banti's disease in the past twenty years, not only is its etiology undetermined, but it is still an open question whether it is a disease due to a specific cause or is merely a fairly constant symptom-complex. Banti attempted to demonstrate microorganisms in the blood and viscera of this disease, but failed, as he did also in his various attempts to reproduce the disease in lower animals. He insisted, nevertheless, that the splenic enlargement was primary and due to an unknown infectious agent localized in the spleen. Recently Gibson¹⁴¹ has reported the finding of a streptothrix in the spleens of certain cases resembling Banti's disease, and Yates, Bunting, and Kristjanson⁴⁸¹ have found diphtheroid organisms in several such spleens. As these findings have not been

confirmed, however, and as the great majority of investigators have been unable to isolate a causative organism (Senator,⁴⁰⁵ Sippy,⁴¹⁰ Zancan,⁴⁸³ etc.), the demonstration of an actual infecting agent must be considered as still lacking. Suggestive evidence, however, was recently brought forward by Hollins,¹⁸³ who by repeated subcutaneous injections of *Bacillus coli* was able to produce in the rabbit a distinct splenomegaly with moderately severe anaemia similar to that of Banti's disease. No haemolytic body or living microorganisms could, however, be demonstrated at autopsy. Banti's later view is that the infectious agent is brought to the artery either as a direct toxin or as a substance which is changed by an actual splenic metabolism into a splenotoxin. The earlier changes are therefore to be found in the neighborhood of the follicular arteries, and later in the pulp, splenic and portal veins, culminating eventually in the liver changes of the third stage. The symptoms, according to Banti, are due to general toxæmia, and the anaemia to a depression of bone-marrow activity rather than to excessive haemolysis. As the degenerative changes in the spleen are too far advanced to permit conclusions as to such a sequence of events, this theory has never been confirmed by experiment or observation. The intimate relationship between the spleen and liver renders intelligible a possible pathology of the third stage, especially as Mallory²⁷¹ and, later, Breccia⁵⁶ have shown that injury to the spleen is followed by focal necroses in the liver. Banti has objected to suggested etiological relationship of intestinal disturbances, despite the many cases in which digestive disturbances are known to precede or usher in the disease. The fact

PLATE IV



Histology of spleen of early Banti's disease.

that the spleen is involved earlier than the liver would point to a haematogenous rather than an enterogenous toxin, and the unquestioned improvement that usually follows splenectomy indicates that the altered spleen is in some way an important pathogenetic factor. This is still further emphasized by Umber's⁴⁴⁴ unique observation. A boy, fifteen years of age, was splenectomized for Banti's disease, and during the operation a small piece of the enlarged liver was excised for histological examination and a distinct peripheral infiltration of the lobules found. Later the liver returned to normal size, a strong indication of the splenogenous origin of the hepatitis, which, if undisturbed, should have progressed to the usual cirrhosis.

Trauma to the spleen has been offered as a causative factor in some cases (Armstrong¹²), while another group of authorities consider Banti's disease merely a syndrome, which a great variety of causes are capable of producing (Albu,⁵ Isaac,¹⁹³ Luce,²⁵⁶ Neuberg,³¹⁴ Seiler⁴⁰²). There is no question that various conditions, as an atypical cirrhosis of the liver with early prominence of splenic signs, syphilis, or primary endophlebitis or thrombosis of the splenic or portal vein can produce a picture which cannot be distinguished from that of Banti's disease (Edens,¹⁰¹ Goldman¹⁴⁸). As endophlebitis or thrombosis is a frequent accompaniment of true Banti's disease, it is but natural that the symptomatology of the two conditions should be confounded. Banti himself called attention to the frequency of these changes in his original descriptions, but considered the splenic changes primary. Other authors consider that when the endophlebitis or thrombosis is primary certain characteristic symptoms will often point toward a proper differential diagnosis. Warthin⁴⁶² in-

sists that an infectious thrombophlebitis of the portal or splenic vein is the essential feature of the symptom-complex, whereas the upholders of the separate-entity theory claim that such changes are secondary to the changes in the spleen or may be absent altogether (Ziegler⁴⁸⁶). In certain cases of syphilis of the liver, also, the splenomegaly, anaemia, and history of haematemesis may be so prominent that the picture of Banti's disease is very closely simulated (Osler³²³).

When a consideration of the above features allows a differentiation to be attempted it would seem advisable to consider such cases as pseudo-Banti's disease, and to restrict the use of the term true Banti's disease for those cases in which no such etiological factor is apparent.

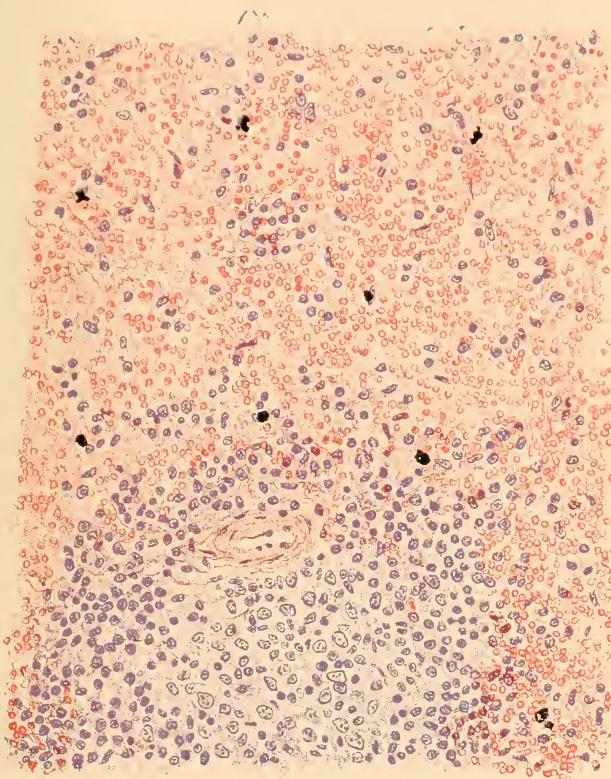
The nature of the splenic enlargement has also given rise to several hypotheses. The view that it is spodogenous can be ruled out, on account of the absence of histological evidence to support it, and Barr's³⁶ theory of splenic congestion due to splanchnic vasomotor paresis needs merely to be mentioned. The prevailing opinion is that the enlargement is due to a chronic inflammatory process, which in turn results in an increased functional activity (increased haemolysis), with a resultant anaemia. This view is in accord with Botazzi's haemocatatonistic and Banti's haemolytic theories. Haemolysis is considered by Harris and Herzog to be due to an erythrolytic enzyme elaborated by the hyperplastic endothelial cells, and by Lintvarew to an increase in the erythrophagetic action of the spleen-cells, the resultant fibrosis being due to the chronic irritation of the products of red-cell destruction. The absence in the circulating blood of signs of bone-marrow

PLATE V



Histology of spleen of late Banti's disease.

PLATE VI



Histology of spleen of congenital haemolytic icterus.

activity points, however, to a diminished activity in blood formation, but against this is the increased urobilin elimination as evidence of increased blood destruction. The probable existence of a splenic hormone to the bone-marrow and its disappearance in splenic disease can be invoked to explain the greater anaemia when the splenic tissue has been largely replaced by fibrosis; but, on the other hand, the improvement in the blood picture that follows splenectomy suggests the removal of a pernicious haemolytic activity on the part of the spleen.

PATHOLOGY.—In the pathological histology of the disease there is nothing specially distinctive. The enlarged spleen, as a rule, shows an increased amount of fibrous tissue in the capsule and reticulum, usually characterized as “fibroadenie” (that is, increased fibrous tissue, but retaining an adenoid appearance), and involving both pulp and follicles. The Malpighian follicles, especially in the later stages, are small and scarce; in the earlier stages they may be hyperplastic and the “fibroadenie” be absent. Macrophages, increased amount of pigment, and other evidences of increased blood destruction are usually found. The changes in the liver are those of an ordinary periportal cirrhosis.

In summarizing, one might say that, although the etiology of true Banti's disease is unknown and may well be from several sources, evidence points to the close causative relationship of the spleen. One would not expect the removal of a largely fibrotic organ to be attended with marked somatic changes, and it is precisely in the earlier stages of the disease in which splenectomy has proved most beneficial.

VON JAKSCH'S DISEASE

The anæmia infantum pseudoleukæmica of von Jaksch^{198a} is in all probability not an independent condition, but represents an atypical response of the infantile haemopoietic system to one or other of the primary diseases of the blood (leukæmia, pernicious anæmia, the secondary anæmia of rickets, syphilis, Banti's disease, or the formerly unrecognized types of haemolytic jaundice). As a rule, a high-grade anæmia, with blood picture somewhat resembling pernicious anæmia, appears in infants of one or two years. Leucocytosis, especially of the small lymphocytes, is a frequent response to any form of anæmia in infancy and childhood. The smooth, hard spleen is conspicuously large, while the liver, in contrast to the liver of leukæmia in childhood, is very slightly enlarged. Aschenheim and Benjamin^{18a} have found rickets present in all of a series of such cases examined by them, and suggest the name "Rachitische Megalosplenie" for this condition. Von Jaksch also associated rickets with this disease. Giffin¹⁴⁴ and others, on the other hand, consider the true v. Jaksch's disease to be nothing more than the infantile form of splenic anæmia. (Banti's disease.) That von Jaksch's disease is being less and less regarded as a separate disease is shown by the gradual disappearance of the name from the text-books.

HÆMOLYTIC JAUNDICE

The acquired, Hayem-Widal, and congenital or familial, Chauffard-Minkowski types of haemolytic jaundice with splenomegaly are frequently grouped by English and American authors under such titles as "haemolytic jaundice" (Thayer⁴³¹), and "chronic family jaundice"

(Tileston⁴³⁵). As the two forms possess several rather important and characteristic differences, it is deemed advisable to follow the continental custom and consider them as independent conditions. Search for their true etiology and pathogenesis (as yet unknown) is more apt to be stimulated under such an arrangement than if they are grouped together. Furthermore, Widal, Abrami, and Brulé⁴⁷⁰ experimenting with toluylenediamine, have offered evidence to indicate, in their opinion, that the two types are of different origin.

SYMPTOMATOLOGY.—The points of differentiation we will present after outlining the historical development of our general knowledge of haemolytic jaundice. Although Murchison,³⁰⁸ Wilson,⁴⁷⁴ and others had previously described cases of chronic jaundice occurring in several members of a family (in Murchison's case splenomegaly is not mentioned), it was the more complete description of Hayem,¹⁷¹ in 1898, that first established the condition as a clinical entity. The clinical picture of the five cases analyzed by him was as follows: All five exhibited a chronic jaundice, with the presence of bile-pigment in the blood-serum, but not in the urine (*i.e.*, acholuric icterus). The other signs of obstructive jaundice, such as itching, bradycardia, and clay-colored stools, were also lacking. A distinct anaemia, the red-cell count varying from 1,000,000 to 3,000,000, was present in all. Very large, hard spleens were found in each case, and slight enlargement of the liver was also noted. Exacerbations were frequent, and during these the jaundice deepened and bile appeared in the urine. The importance of these exacerbations was emphasized by Widal, who termed them "crises of deglobulization," and considered them highly characteristic of the acquired form.

In severe cases the blood count fell below 1,000,000 and haemoglobin appeared in the urine. In all five of Hayem's cases the family history was negative, and in three the jaundice was stated to have appeared first in adult life.

Two years later Minkowski²⁹¹ described a similar disease occurring during three generations in eight members of one family. This, the congenital form, it is now known, is commoner than the acquired form. In addition to the symptoms presented by Hayem's cases, an increased amount of urobilin was noted in the urine. Autopsy revealed no cirrhosis of the liver or obstruction of the bile-passages. The spleen showed a diffuse hyperplasia and hyperæmia. Pigment deposits were numerous in the kidneys and in the centres of the liver lobules.

The next important contribution to the clinical picture of these diseases was made by Chauffard,⁷² who showed in the congenital type that the resistance of the red blood-cells to hypotonic salt solution was much diminished.

Increased number of microcytes and of reticulated red cells by methods of vital staining were found by Chauffard⁷² in the congenital or familial type, and their presence later confirmed also in the acquired type.

Another diagnostic method, the auto-agglutination test, is advocated by Widal, Abrami, and Brûlé.⁴⁷¹ They have found it always positive in the acquired form and always negative in the congenital or familial type. However, in Micheli's²⁸⁹ carefully studied case of the acquired type this test was also negative. Isohaemolysins have occasionally been found in both types (Micheli,²⁸⁹ Hopkins¹⁸⁴), but are not supposed to possess any pathological significance. Although both types of haemolytic jaundice usually

run a chronic course, Gaisböck¹³⁶ has shown that an acute malignant form may occur that is fatal in a few months.

Therefore the cardinal symptoms of the two types of haemolytic jaundice with splenomegaly are found to be a chronic enlargement of the spleen, existing with an acho-luric, non-obstructive jaundice, and anaemia, frequently paroxysmal in character and varying in intensity. Increased blood destruction is indicated by increased urobilin in the urine, and various characteristic changes are found in the blood. The red cells show diminished resistance to hypotonic salt solution, increased number of reticulated cells with vital staining, and in the acquired form the phenomenon of auto-agglutination of the red corpuscles. The blood-serum rarely contains auto- or isohæmolysins.

We have purposely postponed until now a consideration of the differentiation of the acquired and familial types and their relation to other conditions, such as those suggested by Gilbert¹⁴⁶ and Banti.³¹ The fact that in the acquired group the disease is definitely acquired in adult life, whereas in the other there is a family history of the same trouble, is not in itself sufficient to warrant the distinction of independent disease pictures. There are, however, other features which tend to differentiate the two types. In the congenital form the subjects, as Chauffard puts it, "are more icteric than sick." Frequently they come for treatment for other conditions and consider the chronic jaundice as a family idiosyncrasy not interfering with perfect health. The acquired form, on the other hand, is usually ushered in with a definite attack of illness; the anaemia becomes much more grave, sometimes as low as 1,000,000, and the patient is distinctly more anaemic than jaundiced. In Decastello's⁹¹ case, which was greatly im-

proved by splenectomy, the red-cell count had previously fallen to 800,000.

An analysis of 159 cases of haemolytic jaundice in which blood counts are available shows that 55 belong to the acquired type and 104 to the congenital or familial type. Of the latter, only 23 failed to give a positive family history; but of the 81 remaining cases the disease in 36 developed after birth. The term "familial" would therefore seem preferable to that of "congenital," unless a third variety is to be considered. The average red-cell count of the 55 acquired cases is 2,032,000, the counts ranging from 510,000 to 4,500,000. Counts below 1,000,000 are recorded in ten cases; below 2,000,000 in 27 cases, and over 4,000,-000 in only four cases. The average count of the 103, congenital and familial, is 3,340,000, the counts ranging from 1,800,000 to 5,700,000.* No counts are recorded below 1,000,000; eight below 2,000,000, and 25 above 4,000,-000. If this group is subdivided, the average of the familial cases is 3,281,000; of the congenital, 3,543,000. These figures show that there is a more marked anaemia in the cases of the acquired type than in either the familial or congenital types.

Widal and his pupils claim that the auto-agglutination test is only positive in the acquired form, and consider this as important evidence that the two diseases have fundamentally different origins. Attention has already been called to the differential importance of Widal's "crises of deglobulization," but it must be admitted that marked

* One familial case reported by v. Krannhals showed 1,000,000 red cells in a single count, but, as the haemoglobin was between 55 and 65 per cent., the accuracy of the count is questionable.

fluctuations in the degree of blood destruction are present also in the familial type.

Numerous reports are at hand of a condition apparently identical with the acquired form, but following attacks of malaria, syphilis, and other infections. In such cases the signs of excessive blood destruction usually disappear when the underlying cause is successfully treated. The familial form, on the other hand, appears more as an inherited dystrophy of the haemopoietic system, rendering the red blood-cells more easily destructible. On this basis Chauffard at first strongly advised against splenectomy in this type, but subsequently cases have shown such improvement after this treatment that it would seem as if the removal of this site of blood destruction was advisable, whether or not it is the primary seat of the trouble. It must be noted, however, that after Kahn's²⁰⁶ and Roth's³⁸⁶ successful splenectomies in the familial type the resistance of the red cells failed to return to normal. The congenital type with negative family history, grouped with the familial type by most authors, offers no definite ground for differentiation from the acquired form. The mere fact that the disease has already made its appearance at birth is of itself not of fundamental importance if there is no history of similar trouble in the family. If the familial form (which, as a matter of fact, is usually, though not always, congenital) were placed in contrast to the acquired form, the time of onset, as indicated by the term congenital, might well be disregarded. The possibility of an early acquisition of the disease is shown in the case reported by Benech and Sabrazés.⁴¹ With a negative family history, a suckling is supposed to have acquired the disease from her wet-nurse, who, together with her two children, had

a chronic haemolytic jaundice. Certain authors consider the acquired and congenital types as identical. Hynek,¹⁹¹ for instance, bases his opinion on two cases observed by him: in one a mother acquired the disease after childbirth, whereas in her child it appeared congenitally. Plehn³⁵⁵ reports a case appearing congenitally in father and daughter, but not until the twenty-sixth year in the case of a son. Benjamin and Sluka⁴² observed three cases in one family, two appearing congenitally and one in adult life. Many of these cases could undoubtedly be harmonized if the time of onset were disregarded.

In favor of the identity of the two types (acquired and congenital) it must be admitted that a series of cases could be selected in which many grades between the two types would be represented. It is obvious that the familial type must at one time or another have originally been acquired. In such an event the difference in severity between the acquired and familial still holds. Thus both in Roth's³⁸⁶ and Bychowski's⁶⁴ cases the disease in the parent who acquired it was severe, while in the children who inherited it it was of the usual mild type. Such facts, however, would not indicate a fundamental difference in the nature of the two diseases.

In the small number of cases already accumulated various atypicalities have been reported. Thus, Lommel's²⁵² and Claus and Kalberlah's⁷⁹ cases of the familial type and Mosse's³⁰⁴ and Tixier's⁴³⁶ cases of the acquired type failed to show any change in resistance of the red cells (either washed or the whole blood), though other evidences of increased blood destruction were present. In a few cases an acholuric jaundice with splenomegaly has existed together with polychythaemia instead of anaemia. Chauffard

and Vincent⁷⁶ and Roth³⁸⁷ have recently described a form in which haemolysins are present in the blood, and such cases appear to occupy a position midway between haemolytic jaundice and paroxysmal haemoglobinuria "a frigore." Gilbert¹⁴⁶ and his pupils have published numerous reports since 1900 on similar conditions under the name of "cholemie familiale." They have at least served to call attention to the fact that variations may be found in the amount of enlargement of the spleen and liver. It is questionable, however, if it is necessary, as they have frequently done, to consider each atypical form as a separate condition to be dignified with a separate name. As many of their cases were described before the various haematological methods above described had come into vogue, and are frequently reported in *résumé*, it is difficult to determine conclusively whether or not they should be considered as belonging to the types under discussion. Their theory of hepatic origin, through an infectious angiocholitis, they later abandoned in favor of Chauffard's idea that the primary change was in the blood.

Another occasional variation is in the presence of all symptoms of increased blood destruction but an absence of icterus. This Chauffard describes in a family in which the mother is a typical haemolytic icteric, while the eighteen-year-old son presents all the signs of the disease except jaundice.

When the anaemia is grave the blood may present a picture indistinguishable from pernicious anaemia (v. Stejskal⁴¹⁵). Chauffard considers that there is an icteric form of pernicious anaemia which, when accompanied by diminished resistance and reticulated red cells, represents the least compensated form of haemolytic icterus. Widal

and Weissenbach⁴⁷² have also reported a case of this type. In the usual Biermer type of pernicious anaemia, icterus, it will be recalled, is absent and the resistance of the red cells increased.

Banti³² has recently proposed the name "haemolytic splenomegaly" for a type of the disease which on analysis is indistinguishable from the acquired form of haemolytic jaundice. Both the cases described by him exhibit a chronic anaemia, with long-standing splenomegaly, subicterus, diminished resistance of the red cells, increased number of reticulated cells, and urobilinuria. In a later study³³ of seven cases of this condition he further subdivides "haemolytic splenomegaly" into regenerative and degenerative groups, the difference depending chiefly on periods of relapse and remission in the former type, with greater evidences of regeneration in the blood picture. The beneficial effects of splenectomy—abolishing the anaemia and changing the resistance to normal in one case and even changing an aplastic into a reacting bone-marrow—lead him to ascribe a primary rôle in this disease to the spleen. It must be remembered, of course, that removal of the normal spleen indirectly causes an increase in resistance of the red cells. Banti claims never to have seen Widal's crises of deglobulization in these patients, and emphasizes the presence of a relative lymphocytosis, but this would hardly indicate a different disease. As to the choice of name, Banti's name is open to the same objection as the older one; namely, that splenomegaly may be absent in some cases (Le Gendre,²⁴³ Pick,³⁵³ Gilbert and Lereboullet,^{146a} Benjamin and Sluka's third case, and Marchiafava and Nazzari,²⁷⁶) just as jaundice is in others. A name indicating increased blood destruction, such as "haemo-

lytic hypersplenism," without including individual features, would be less open to objection; but in the meantime, until hypersplenism can be demonstrated, the name sanctioned by usage is preferable.

PATHOGENESIS.—As I have already indicated in the discussion of the differentiation of the two types, a hepatic or luetic etiology has been largely discarded. Such etiology is, however, still occasionally reported (Bernard, de Berumann, Gelle), as is also that of malaria (Socquepee) and ankylostomiasis (Darre, Parisot, and Fairise). The two prominent views, however, are (1) that the primary lesion is in the blood—a dystrophy of the red cells; or (2) either primarily or indirectly in the spleen—an exaggerated haemolytic activity. Widal and his school, the extreme supporters of the former view, consider that the congenitally weak red blood-cells are destroyed in the circulation and their remains taken up by the spleen, causing a spodogenous tumor, and by the liver, kidney, and bone-marrow, as shown by the excessive pigment deposits found in these organs at autopsy. This view was supported by Vaquez,⁴⁵⁰ von Stejakal, Benjamin and Sluka, Aschenheim,¹³ and Weber and Dorner.⁴⁶³ The chief objection to it is that it completely ignores the great improvement following splenectomy. A primary increased haemolytic activity of the spleen as the source of the malady was first proposed by Minkowski and supported by von Krannhals and Chauffard. Its latest adherent, Banti, as I have previously stated, considers that the pathological spleen not only is spodogenous, but actively destroys increased numbers of cells and prepares others for destruction. Though based on incorrect and inadequate experimental evidence, this attractive combination of the splenogenous and haemo-

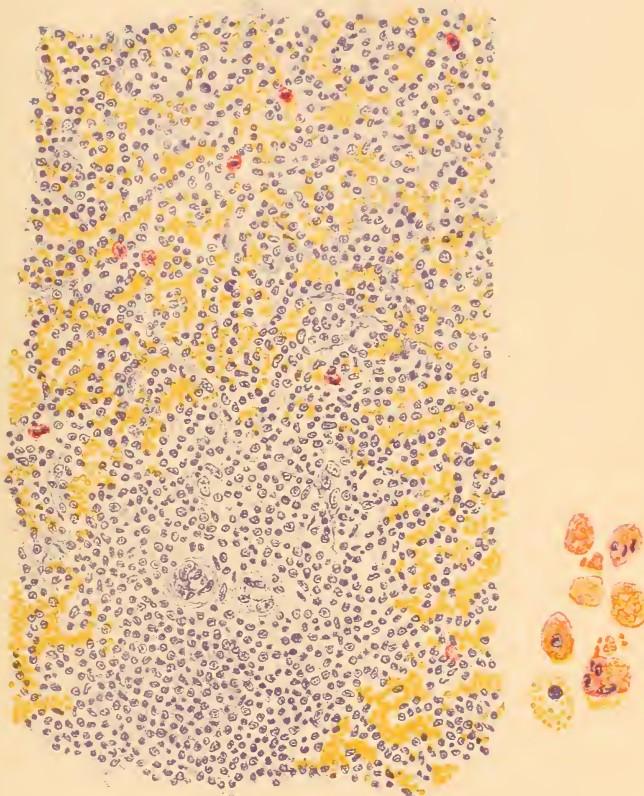
catastrophic theories at present seems most plausible. Although it is not possible to demonstrate haemolysis in the normal spleen, we cannot exclude the possibility that it exists and that it is active in some diseased conditions of the organ. Extracts from the spleens of Antonelli's,⁹ Kahn's,²⁰⁶ and Robertson's³⁷⁹ cases, however, failed to show any haemolytic activity *in vitro*.

Whatever the source of the increased blood destruction, there results an increased amount of free haemoglobin to be gotten rid of. Most authorities believe that this is changed by the liver into bile in excessive amounts, and that the viscous and highly pigmented bile clogs the bile-capillaries, is reabsorbed into the blood, and thus causes a "pleiochromic icterus." Recent investigations by Whipple,⁴⁶⁸ however, show that bilirubin can be formed by the action of the endothelium of blood-vessels entirely isolated from the hepatic circulation. If this be true, a haematogenous icterus in the narrower sense can be accepted. That the jaundice is not due to gross obstruction is proved by the facts that such obstruction has never been found, that the stools are of normal color, and that the urine does not contain bilirubin.

It is interesting to note that in Banti's aplastic or anæmopoietic case splenectomy started the formative powers of the bone-marrow, as shown by the appearance of normoblasts in the circulating blood. This would indicate that with the spleen was removed a toxin that had inhibited blood formation, and from this it could be argued that the decreased resistance of the red cells of haemolytic jaundice is due, not to a haemocatatonic action of the spleen, but to an indirect injury to the bone-marrow.

Pathological study of the comparatively few cases of

PLATE VII



Spleen of pernicious anæmia. Detail drawing shows phagocytosis of red cells and blood pigment.

haemolytic jaundice that have come to autopsy or splenectomy has yielded little in the way of establishing a constant and characteristic pathological picture. I have collected descriptions of seven spleens obtained at autopsy and of eight obtained after splenectomy. An analysis of these appears in Table LXII.

It will be seen that the chief characteristic of both types of the disease is the marked congestion of the splenic pulp and splenic sinuses, but this is, of course, found in many other conditions. The Malpighian follicles, the capsule, and trabeculae are frequently left unchanged; and, while pigment deposits and macrophages have usually been found to be increased, in the case reported from this laboratory this was not found to be true. In all cases in which the bone-marrow was examined at autopsy it was found to be red.

PERNICIOUS ANAEMIA

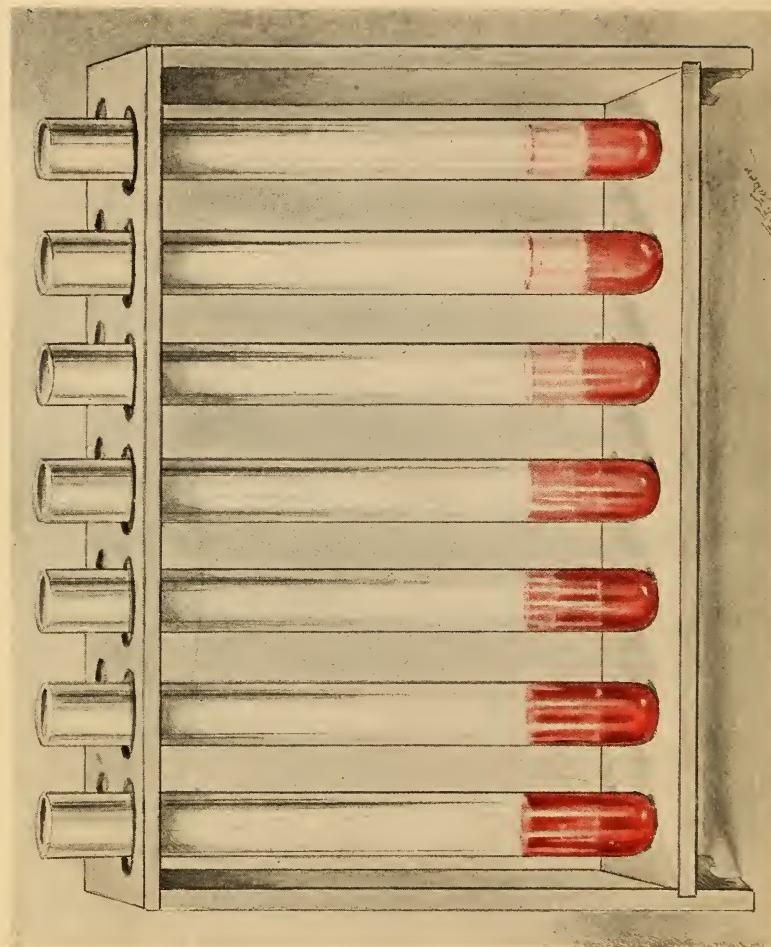
This disease is mentioned briefly here, not as an example of splenomegaly, but because the recent tendency to treat it by splenectomy brings it into relation with the theory of hypersplenism.* This latter theory has been put forth prominently in recent years by Eppinger¹⁰⁴ and King,²¹³ who consider that the amount of haemolysis in a given case is in definite relation to the amount of unsaturated fatty acids and the amount of urobilin in the faeces. They found both these increased not only in haemolytic jaundice, but also in pernicious anaemia, hypertrophic cirrhosis, and catarrhal jaundice. For example, the unsaturated fatty acids, as may be seen in Table LXIII, rise from a normal (iodine number) of 80 or 90 to 188 to 273

* For summary of recent literature on this subject see Vogel.⁴⁵⁶

TABLE LXII
PATHOLOGY OF HæMOLYTIC JAUNDICE

Author	Type	Based on	Weight of spleen gms.	Capsule and trabeculae	Pulp	Sinus	Malloryan foli- cles	Macro- phages in spleen	Pigment in liver	Bone marrow	Miscellaneous
Minkowski...	Familial	Autopsy	675	Normal	Congestion	Congestion	Increase	Increase	Red	Much pigment in kidneys
Veguer and Giroix	Familial	Autopsy	850	Normal	Marked congestion	Congestion	Normal	Increase	Normal	Red	
Gandy and Brûlé	Familial	Autopsy	750	Normal	Marked con- gestion	Congestion	Normal	Slight in- crease	Increase	Red	
Marchiafava	Familial	Autopsy	270	Thick	Neutrocytic	Increase	
Oettinger	Acquired	Autopsy	575	Normal	Marked con- gestion	Congestion	Small	Increase	Increase	Red	
Micheli	Acquired	Autopsy	850	Thick	Congestion	Congestion	No germ centres	Increase	Increase	Red	
Kahn 1	Familial	Operation	970	Normal	Congestion	Congestion	Normal	Increase	Slight in- crease	
Kahn 2	Familial	Operation	1170	Thick	Congestion	Congestion	Few	Increase	
Umber	Acquired	Operation	1300	Normal	Congestion	Congestion	Pigmented and congested	Increase	
Antonelli	Acquired	Operation	1000	Normal	Congestion	Congestion	Normal	Increase	Increase	
Fiori	Acquired	Operation	1000	Normal	Marked con- gestion	Congestion	Normal	Increase	Increase	
Banti 1	Acquired	Operation	1580	Normal	Marked con- gestion	Congestion	Normal	Increase	Increase	
Banti 2	Acquired	Operation	970	Normal	Marked con- gestion	Congestion	Normal	Increase	Increase	
Gaisböck	Acquired	Autopsy	?	Normal	Not con- gested	Reticulum increased, pulp cells decreased	?	Increase	Increase	Red	
Goldschmidt, Pepper and Pearce	Congenital	Operation	640	Normal	Not con- gested	Congestion	Hyaline thickening of central artery	Notseen	No in- crease		

PLATE VIII



Test for resistance of erythrocytes. Salt solution (NaCl), varying in strength from 0.3 to 0.5 per cent. Note that the tube at the left of the series shows complete haemolysis and that at the right shows no haemolysis. Those in between show a progressing amount of partial haemolysis as indicated by the changing depth of coloration of sediment and supernatant fluid.

in pernicious anaemia. As they had found experimentally that removal of the spleen in the dog caused a great drop in the iodine number and was associated with an increased resistance of the red cells and lessened tendency to haemolysis, they favor splenectomy in this type of anaemia. Eppinger's theory of the pathogenesis of pernicious anaemia is interesting, but not convincing. On histological

TABLE LXIII

ANALYSIS OF BLOOD FAT IN ANÆMIA AND OTHER CONDITIONS * (MODIFIED FROM TABLE OF EPPINGER¹⁰⁴ AND KING²¹³)

		Total fat gms.	Choles- terin gms.	Choles- terin ester gm.	Iodin number
Normal.....	1	5.38	0.76	0.52	90
Normal.....	2	5.90	0.86	0.57	79
Pernicious anaemia.....	1	7.34	0.56	0.72	188
Pernicious anaemia.....	2	8.40	0.32	0.01	213
Pernicious anaemia.....	3	9.37	0.14	0.05	273
Hæmolytic jaundice.....	1	5.43	0.49	0.41	326
Hæmolytic jaundice.....	2	6.33	0.85	0.31	258
Cirrhosis of liver.....	1	9.35	0.91	0.31	125
Cirrhosis of liver.....	2	3.94	0.30	0.13	309
Cirrhosis of liver.....	3	7.36	0.56	0.12	82
Catarrhal jaundice.....	1	5.70	0.50	0.23	187
Catarrhal jaundice.....	2	5.40	0.39	0.44	123
Catarrhal jaundice.....	3	5.60	0.48	0.67	122
Obstructive jaundice.....	1	10.63	1.07	0.27	88
Obstructive jaundice.....	2	7.92	0.61	0.49	139
Secondary anaemia, carcinomatous stomach.....	1	6.39	0.71	0.52	36
Secondary anaemia, carcinomatous oesophagus.....	2	6.97	0.38	0.44	101
Nephritis.....	1	8.24	0.56	0.38	22
Nephritis.....	2	15.89	1.26	0.07	69
Polycythaemia.....		5.93	1.09	0.08	273
Purpura.....		8.50	0.86	0.63	224

* Figures are for 1000 c.c. of blood.

examination of the spleen he found thickened walls in the arterioles and intense congestion of the pulp. He assumes, therefore, that the blood seeks the path of less resistance through Weidenreich's open capillaries into the pulp, where they are destroyed, presumably by contact with the connective tissue. From this rather fantastic point of view,

splenectomy therefore becomes equivalent to tying off a ruptured blood-vessel.

SUMMARY.—The several diseases described in this chapter not only have sufficiently characteristic and constant symptom-complexes to permit a differential diagnosis to be made, but are also, in all probability, due to different causes, or possibly to a common factor operating in different ways. In a strict sense, none of them should be considered as primary anæmias, though in some it is difficult or impossible to find the “*causa causorum*.” From the aspect of the chief lesion found (namely, the changes in the blood), they may be divided into two groups, in one of which increased blood destruction and in the other impaired blood formation is characteristic. As will be shown in a later chapter, the relative importance of these features has an important bearing on the results produced by transfusion and splenectomy. From this point of view, the anæmia of Banti’s and Gaucher’s diseases is chiefly due to impaired blood formation, while that of the hæmolytic jaundices is due to impaired blood destruction. Finally, in another disease, pernicious anæmia, commonly treated by splenectomy, increased hæmolysis predominates, but is usually accompanied by seriously impaired powers of blood formation.

CHAPTER XII

METHODS OF VALUE IN THE DIAGNOSIS AND PROGNOSIS OF SPLENIC DISEASE

THE numerous points of resemblance or of slight dissimilarity in the several clinical conditions analyzed in the previous chapter demonstrate that in a given case careful study must usually be made before a proper diagnosis can be reached and such studies continued, if the prognosis and effect of treatment are to be properly gauged. An important part of these studies is not only the performance of certain special tests to be described in this chapter, but also the proper accomplishment of the usual history taking, physical examination, and routine blood examinations.

In history taking, emphasis should be laid on a most thorough inquiry into the family history for evidence of disease of a similar nature, either in the present or former generations. Not only should exhaustive search be made into the patient's or parents' past histories for possible underlying or contributory causes, but also the most probable time of onset of the disease must be carefully investigated. Experience has shown that it is in connection with these three points that most defective histories are at fault. The physical examination should always include careful investigation into the presence or absence of jaundice, and the size of the liver, spleen, and lymph-nodes. Evidence of jaundice should be sought not only in the skin and mucous membranes, but by appropriate tests of the urine

and by inspection of the blood serum.* Frequently repeated routine blood examinations should be made. The chief fault in connection with such examinations is that they are not repeated often enough, both before diagnosis has been reached and while the effect of treatment is being studied.

The special laboratory tests to be described in this chapter are all concerned with attempts to study as nearly quantitatively as may be both the nature and degree of the disease process (estimation of amount of blood destruction, changes in the blood-serum and in the resistance of erythrocytes), and the ability of the body to compensate therefor (evidences of blood regeneration). It must, however, always be remembered that the constantly changing factors of blood destruction and blood regeneration are being dealt with, so that conditions may be met with in which: (1) Blood destruction is excessive, but powers of regeneration well preserved (as in haemolytic jaundice); (2) blood destruction excessive and powers of regeneration insufficient (as in pernicious and aplastic anaemia); (3) blood destruction not excessive, but powers of regeneration insufficient (as in Banti's and Gaucher's disease), with an infinite number of intermediate grades. Such considerations are further complicated by the fact that the bone-marrow response may be considerable, but pathological in type, as in remission stages of pernicious anaemia. It must also be remembered that, although fairly accurate indirect methods exist for estimation of the amount of blood destruction,

* Blood should be withdrawn from ear or finger-stab into a pointed glass tube of small calibre, which is then sealed by heat and allowed to stand for several hours.

the study of blood regeneration is still largely qualitative.

The tests referred to may be considered under the following heads:

- A. Resistance of erythrocytes.
- B. Evidences of bone-marrow activity (reticulated cells; nucleated forms, platelets).
- C. Agglutinins and haemolysins in the blood-serum.
- D. Urobilin excretion.
- E. Protein, uric-acid and iron metabolism.

A. RESISTANCE OF ERYTHROCYTES TO HYPOTONIC SALT SOLUTION

The resistance (fragility) of erythrocytes to various fluids was first studied by Malassez²⁶⁷ in 1873, and the mechanism of the destruction of the red blood-cell has been more or less imperfectly understood since the time of Hamburger's¹⁶⁵ investigations on osmosis of body fluids. Although hypotonic salt solution in varying strengths has been the most commonly used in clinical tests, saponin, snake venom, bacterial haemolysins, and specific haemolytic immune serums have also been employed either as clinical tests or in attempts to explain the mechanism of the destruction of the erythrocyte. As a general rule, if the resistance of erythrocytes is increased or decreased to one of these agents, it will be so to all, but occasional exceptions have been noted. Thus in obstructive jaundice and in pernicious anaemia it has been claimed that the resistance of erythrocytes to saponin is diminished, whereas it is increased to hypotonic salt solutions. In our own work, on the other hand (see page 42), we have found that in animals under various experimental conditions the changes

in resistance to saponin and hypotonic salt solution were always parallel. On account of such possible divergences, however, it is advisable, for the present at least, to confine routine tests to the hypotonic salt solution method.

This method depends on the simple principle that erythrocytes can remain for some hours in isotonic salt solution without damage, whereas when placed in distilled water they are very quickly haemolyzed, the haemoglobin being "laked out" of the corpuscular stroma. If, then, suitable intermediate strengths of solution are arranged, it can be determined in just what strengths of salt solution partial haemolysis occurs, and at which point complete haemolysis first occurs. The various ways of applying this test have been considered in detail by Ribierre,³⁷⁰ the method finally adopted by him being as follows: Glassware should be sterilized and the chemically pure sodium chloride should be desiccated before preparation of stock solutions, to get rid of the "water of interposition." In normal cases nine small tubes are arranged in strengths varying as follows: 0.50, 0.46, 0.44, 0.42, 0.40, 0.38, 0.34, 0.32, 0.28 per cent. NaCl. (If it is found that haemolysis occurs at 0.50 per cent., a second test is made with solutions of the strengths 0.60, 0.56, 0.52.) The finger of the patient is carefully cleansed, pierced in the usual manner, and blood sucked into a pipette to a mark denoting one-fiftieth of the content of the pipette (about 2 c.c.). It is then filled with the appropriate strength of salt solution, mixed and emptied into one of another series of small tubes, avoiding as much as possible the admixture of air, and the operation is repeated through the series of tubes. These are then covered with rubber caps, allowed to stand five minutes, centrifuged for one and one-half minutes, and the results observed. Ribierre

has found that, although after twenty-four hours the amount of haemolysis is slightly increased, there is no appreciable difference between a five-minute and a three- or four-hour period. Many observers still consider it necessary to defibrinate and wash the erythrocytes; but this not only requires greater quantities of blood and considerably increases the difficulty of the examination, but also to a slight extent mechanically injures the cells, so that a slightly lessened resistance is found. To be sure, Widal, Abrami, and Brûlé showed that in some cases of the acquired form of haemolytic jaundice with apparently normal resistance fragility would be demonstrated if the cells were washed free of plasma. Later work, however, both in this laboratory and elsewhere, has tended to show that any change, when present, is in the cells themselves.

In our work in this laboratory, both on patients and animals, the use of a mixing pipette has been found unnecessary, the measurement of many drops to each tube tedious, and sedimentation for one hour has proved preferable to centrifugalization. The test is therefore performed as follows:

Stock solutions of sodium chloride are prepared as above described in strengths varying by 0.02 per cent. from 0.20 to 0.70 per cent. If kept tightly stoppered these may be used for several months, but should be renewed earlier if control tests show any change in concentration of solutions.

A series of twelve or more tubes containing 1 c.c. of different strengths of hypotonic salt solution are then arranged, varying by 0.02 per cent. from 0.25 per cent. to 0.60 per cent. (or even stronger, if diminished resistance

is suspected). Into each one drop of whole blood is introduced and the tube gently shaken. If the drops have been of different size, slightly more blood may occasionally have to be added until the color is the same in all. After standing two hours at room temperature, in the stronger solutions in which no haemolysis has occurred, the unchanged corpuscles at the bottom of the tube will be overlaid with colorless salt solution. In the weakest solutions all corpuscles will have been haemolyzed, forming a transparent red solution. In the intermediate tubes can be noted the point at which haemolysis begins and at which it is complete.

In normal cases haemolysis begins at about 0.45 and is complete at 0.35 per cent. In a case of the familial type recently examined haemolysis began as high as 0.7 per cent. and was already complete at 0.475 per cent. In most other anæmias the resistance is nearly always more or less increased, depending on the severity of the anæmia. Haemolysis may not begin until solutions as low as 0.36 are reached, or may not be complete before 0.24 or 0.26 per cent. In pernicious anæmia, while the resistance is usually greatly increased, cases have been reported in which the resistance is normal or even diminished, so that there is a marked resemblance to haemolytic jaundice.

B. EVIDENCES OF BONE-MARROW ACTIVITY

1. *By Vital Staining. Skeined or Reticulated Cells.*—Amidst the confusion that surrounds the subject of stainable granules in the erythrocytes, a few facts are generally accepted by hematologists. One such is that a basophilic reticulation is demonstrable by the methods of vital stain-

ing in a very small percentage of normal cells, but in greatly increased numbers in various diseased conditions of the blood. This special method of examination was first described by Chauffard and Fiessinger,⁷³ in 1907, in connection with their study of a case of congenital haemolytic jaundice, in which condition the reticulated (granulous or skeined) erythrocytes are very much increased. These authors used Pappenheim's pyronine methyl green stain (equal parts of saturated aqueous solutions of pyronine and methyl green, prepared at least several days before use, and filtered just before using), but also suggest the use of neutral red in isotonic solution, while Widal recommends polychrome methylene blue, and we have found the best results with brilliant cresyl blue.

This simple test is performed as follows: A few grains of the stain are dissolved in a perfectly clean, small test-tube in 1 c.c. of normal salt solution, together with one or two small crystals of potassium oxalate, to prevent rouleaux formation.* As the exact strength of the solution is immaterial, it is sufficient to prepare the stain in this way, aiming to get a strength of solution that is just translucent in a test-tube of 1 cm. diameter. A few drops of blood are allowed to flow into this tube, the mixture gently shaken and allowed to stand ten or fifteen minutes. A drop of the sediment is then pipetted off, a fresh cover-slip preparation made and examined under an oil immersion lens. The blue-staining reticulum is easily visible under such conditions, and the percentage of reticulated to non-reticulated erythrocytes estimated. The protoplasm of the erythrocytes is not affected by the stain, whereas the various

* The solution of sodium oxalate in salt solution may be prepared beforehand and kept on hand, if the test is made frequently.

forms of leucocytes, the platelets, and hæmokonium are readily stained and identified. The average diameter of the reticulated cell, according to Chauffard and Fiessinger, is 8.1μ , as compared with an average diameter of 6.3μ for the non-reticulated cell.

The reticulum persists longer than the protoplasm of the erythrocyte under the conditions of this test, but the majority of both will disappear in the course of a few hours. If an accurate comparative count is to be made, the cover-slip should be prepared and examined between fifteen and thirty minutes of the time of obtaining the blood.

In the blood of normal human subjects, reticulated erythrocytes are either entirely absent or constitute but a very small fraction of one per cent. They tend to be slightly increased in any considerable anaemia, but rarely exceed 1 to 4 per cent. Thus Chauffard and Fiessinger found an average of one cell in 500 and one in 400 in cases of tuberculosis, one in 400 and one in 600 in mitral disease, one in 600 in malaria, one in 500 in plumbism, one in 200 in chronic nephritis, and one in 100 and one in 50 in plumbism with nephritis. In a case of tuberculous nephritis with profound anaemia, subicterus, and diminished resistance of erythrocytes, the reticulated forms were considerably increased (2 per cent.). In cases of haemolytic jaundice, on the other hand, the percentages of reticulated forms reach as high as 15 to 20 per cent., and in the case reported in the previous section of this book (page 202) the counts were frequently above 5 per cent. The proportion of reticulated cells varies considerably in different species, these cells in the normal dog being even rarer than in man, whereas in the rabbit they constitute about 2 per cent. of the total number.

PLATE IX



Reticulated red blood-cells. From a case of hæmolytic jaundice

The nature of the reticulated erythrocytes has not yet been demonstrated, but whether the reticula are nuclear fragments of immature erythrocytes, indicating a bone-marrow reaction (as has been generally considered), or are the results of a disease process, their identification is of great diagnostic value, and their diminution or disappearance as the result of treatment may be taken as a sign of good prognostic significance.

2. *By Fixed Smears* (Nucleated forms, Howell-Jolly bodies, etc.)—Although no direct method exists of determining the rate of blood formation, it may be considered as equal to blood destruction (as estimated from urobilin excretion, etc.) so long as the blood counts remain constant. Although even this method fails if the count is rising or falling, nevertheless under all conditions some criteria exist for estimating the effort required of the blood-forming organs (mainly bone-marrow) to maintain the cellular elements of the blood at the level they happen to be. Thus in the stained dried smear of the peripheral blood, stained with any of the Romanowsky group of stains, the presence of nucleated forms may be taken as evidence that the bone-marrow is so hard pushed that it must allow immature forms to appear in the peripheral blood. If the nucleated erythrocytes are megaloblasts, the diagnosis of pernicious anaemia is strongly suggested, but it must not be forgotten that other chronic anaemias, particularly those in which small hemorrhages occur over long periods of time, can also produce megaloblasts in the peripheral circulation. It must also be remembered that in certain rare conditions, such as tumors of the bone-marrow, erythroblasts may appear in the peripheral circulation without indicating excessive blood regeneration.

Howell-Jolly bodies and the Cabot ring forms should, like the reticulated forms, be taken as evidence of the demand on the bone-marrow for increased blood formation and of its ability to respond to the same. The increased number of these and of nucleated forms after blood transfusion or splenectomy (so-called "blood crisis") are similarly taken as the response to the bone-marrow stimulation that these operations cause. These so-called "blood crises" will be considered again in the chapter on the results of splenectomy under the heading of "Pernicious Anæmia," in which condition they have been most carefully studied; but it should be noted that they have also been observed after splenectomy in Banti's disease, hæmolytic jaundice, and other conditions. If absent after splenectomy when the blood count is rising (as in Goldschmidt, Pepper, and Pearce's case), it would indicate that, with the cessation of increased hæmolysis, the bone-marrow is no longer forced to put forth immature forms. If, on the other hand, both blood crisis and improvement in blood count are wanting, it would indicate that an exhausted bone-marrow is no longer capable of responding to stimulation with the production of even these immature forms.

The blood crisis, which so frequently follows splenectomy for splenic disease, has been shown in Chapter II to be practically absent after removal of the normal spleen. This apparent discrepancy is similar to the fluctuations in the red blood-cell count after splenectomy, which falls after removal of the normal spleen, but rises after splenectomy in splenic disease. The most probable explanation of both paradoxes seems to be that in disease the improvement is due to the removal of an agent which both causes excessive hæmolysis and depresses bone-marrow function,

whereas the anaemia following normal splenectomy is due to loss of normal stimulus to blood formation, and, for the same reason, unusual signs of bone activity are lacking.

Polychromatophilia, like the presence of microcytes and poikilocytes in the blood stream (Rous)^{387a} probably indicates degeneration of the erythrocyte rather than the appearance of immature forms. Macrocytes, like megaloblasts, are probably signs of perverted bone-marrow activity. The diminution or increase of the number of such forms, may, however, safely be taken as indicative respectively of amelioration or aggravation of the disease process.

3. Blood Platelets and Leucocytes.—If Wright's theory be accepted, that the blood-platelets are independent elements of the blood formed from the megakaryocytes of the bone-marrow, both leucocytes and platelets⁴⁷⁸ enter into the problem of blood regeneration. The leukopenia and the diminished platelet counts found in many of the diseases here under consideration should therefore be taken as further evidence of deficient blood formation. The increase in blood-platelets observed by Lee, Vincent, and Robertson²⁴¹ after splenectomy, may therefore be considered another factor in the "blood crises" previously described. In disease conditions a gradual change in these elements of the blood in the direction of normal figures should also be taken as a sign of improvement, though not necessarily of permanent improvement.

C. AGGLUTININS AND HÆMOLYSINS IN THE BLOOD-SERUM

1. Auto-agglutinins.—A simple test that Widal, Abrami, and Brûlé⁴⁷¹ have found of value in differentiating the congenital and acquired types of hæmolytic jaundice is that suggested by Brûlé after the method of Pagniez

for the presence of auto-agglutinins in the serum. This test consists merely of mixing one drop of the patient's washed red blood-cells with ten drops of the patient's own serum in a watch-glass. If positive, after some seconds, the mixture loses its homogeneous aspect, visible granules of agglomerated corpuscles are evident on agitating, and finally, after several minutes, a distinct pellicle forms that sinks to the bottom and does not mix with the clear serum on shaking. In doubtful cases the result can be confirmed by microscopic examination. Widal, Abrami, and Brûlé found this test intensely positive in three cases of acquired haemolytic and negative in two cases of the congenital type, and also negative in cases of alcoholic cirrhosis, simple catarrhal jaundice, and other conditions. They state that, while "iso-agglutination" (agglutination of corpuscles by serum of subjects of the same species) is frequently observed (60.9 per cent. of cases, according to Pagniez), auto-agglutination is, so far as they know, never positive except in this condition.

2. *Autolysins, Isolysins, and Heterolysins*.—The haemolytic power of the patient's serum may be tested on his own blood-corpuscles (autolysis), on the corpuscles of other human blood (isolysis), or on the corpuscles of another species (heterolysis) (see Widal and Wiessenbach⁴⁷²). To twenty drops of the patient's fresh serum is added one drop of washed red blood-corpuscles from the appropriate source. The mixture is incubated at 37° for thirty minutes and the existence and degree of haemolysis noted in the color of the supernatant fluid. Tests for heterolysins are of little value, as they are frequently present in normal sera. Comparative tests may be made with tubes containing different amounts of the patient's

serum and the results compared with those in similar tests of known normal blood. While positive tests for autoysis have, so far as I know, never been reported, Chauffard, Widal, and Weissenbach and others have reported cases that at times of exacerbation of the disease give a positive "isolysin" test, indicating the presence in the serum at these times of a free haemolysin. During periods of remission these isolysins were not found. It must, of course, be remembered that Moss^{303a} and others have found that the blood of a certain percentage of individuals is normally isolytic and isoagglutinative to the blood of other individuals of the same species.

D. UROBILIN EXCRETION

It is now generally recognized that in the estimation of the amount of urobilinogen and urobilin excreted in the urine and stools there exists a fairly accurate index of the amount of blood being destroyed in the body. Emphasis has been laid throughout this book on the importance of the study of blood destruction in its relation to the spleen, and it is Eppinger's¹⁰⁴ great service to have brought us to recognize the importance of urobilinogen and urobilin excretion in the stool as the best index of such destruction. Instead of such indefinite criteria as an enlarged spleen, tendency to acholuric jaundice, or the existence of anaemia coincident with active blood formation, Eppinger was led to search for a more definite index, and found it in Charnas's spectroscopic method for the quantitative estimation of the amount of urobilinogen and urobilin in the stool. Although this has been generally replaced by the Wilbur and Addis⁴⁷³ method, it still remains only roughly quantitative and a rather disagreeable and

time-consuming test, so that improved methods are still to be hoped for. Emphasis must be laid, however, on the fact that examination of the stool as well as the urine is essential; for, while urobilin is more apt to be present in the urine when there is an increased amount of blood destruction than in normal conditions, this is in the nature of an overflow through the liver, and may be absent when there are still greatly increased amounts in the stool; and, furthermore, the amount of urobilinuria may be influenced by a poorly functioning liver.

METHOD.—The method for estimating the amount of urobilinogen and urobilin in the stools is described by Wilbur and Addis as follows:

URINE

"The method of collection of the twenty-four-hour urine has a considerable effect on the total spectroscopic reading. The vessel in which the urine is collected should be of dark-brown glass and should be kept in darkness. Thymol crystals should be added, for, even in cases in which no obvious fermentation had occurred, we sometimes found a diminution in the total amount if no preservative was present. After measuring the amount of the twenty-four-hour urine, 10 c.c. are mixed with 10 c.c. of a saturated alcoholic solution of zinc acetate and, after a few minutes, filtered. If a number of urines are being examined at the same time, it is convenient to have test-tubes graduated to 10 and 20 c.c. Ten cubic centimetres of the filtrate are taken and 1 c.c. of Ehrlich's solution* is added. It was found that this amount produced a sufficient concentration

* Paradimethylaminobenzaldehyde, 20 gm.; concentrated hydrochloric acid, 150 c.c.; water, 150 c.c.

of acid in the mixture to give the maximum intensity of the urobilin band and contained enough of the para-dimethylamidobenzaldehyde for the reaction with urobilinogen. The development of the urobilinogen band is not instantaneous. We found that, as a rule, it had attained its full intensity in a quarter of an hour. The action can be greatly accelerated by heating, but this is to be avoided. It is better to wait for an hour before making the reading, and during this time the solution should be kept in the dark. After three or four hours there is a diminution of the urobilin and urobilinogen in filtrates from urines, so the estimation should be made not later than three hours after adding the Ehrlich solution. We found that Citron's hand spectroscope * was the most convenient instrument to use. The filtrate was washed into a graduate and diluted with tap-water until first one and then the other band of light absorption had disappeared when the full amount of light entered the spectroscope, but were still visible when the light was partly shut off. This gives a fairly definite end-point, and we did not find any great variation in the readings made by different persons. It is important, of course, that the light shall be always of approximately equal intensity. We made the readings in a dark room with a tungsten electric bulb, holding the spectroscope close to the source of light. In highly-colored urines one may be in doubt as to whether or not a trace of urobilin is present, for there may be so much general absorption of light as to obscure the urobilin band in the undiluted filtrate. There is no such difficulty with the urobilinogen band, which lies between the red and yellow where there

* This can be obtained from Paul Altmann, Luisenstrasse, Berlin, Germany.

is no marked light absorption. With urines containing bile, if the amount of urobilin is not very large, it is necessary to add some fuller's earth and to leave the mixture standing for some time before filtration. If this is done the urobilin band can usually be read even in the undiluted filtrate. The dilution required gives the value for 5 c.c. of urine. If this figure is multiplied by the number of 5 c.c. quantities in the twenty-four-hour urine, the number of dilutions which would have been necessary if all the urobilinogen and urobilin in the twenty-four-hour amount had been concentrated in a volume of 5 c.c. is obtained. For instance, if in a twenty-four-hour urine measuring 1000 c.c. a reading of ten dilutions for urobilinogen and of twenty for urobilin were made, the total urobilin would be $30 \times 200 = 6000$. We tried to determine the dilution value of definite weights of urobilin prepared from bilirubin, but different preparations varied so much in their dilution value that it was obvious that we were not dealing with pure urobilin, and we abandoned any attempt to express our results in milligrammes of urobilin.

STOOLS

"All the fæces passed in the twenty-four hours were collected in the same receiver, the stools being protected from light. They were then washed into a large graduate and thoroughly ground up with water into a homogeneous paste, and water added to 0.5, 1, or, if necessary, 2 litres, depending on the quantity of stool. After thorough mixing, 25 c.c. were taken and 75 c.c. of acid alcohol (95 per cent. alcohol, 1600 c.c.; concentrated hydrochloric acid, 25 c.c., and water, 800 c.c.) were added. The mixture was then put into a shaker for about half an hour. A consid-

erable number of extractives were tried for removing the urobilin from the stools, but none were found so efficient as alcohol with hydrochloric acid. After thorough mixing in the shaker, an equal quantity of a saturated solution of zinc acetate in alcohol was added and the mixture was filtered. After adding 2 c.c. of Ehrlich's reagent to 20 c.c. of the filtrate, the solution was put aside in a dark place until next day. The addition of zinc acetate is not absolutely essential, but in some cases we observed an intensification of the urobilin band following its use, and it is perhaps an advantage to make the urine and stool readings so far as possible under the same conditions. The development of the urobilinogen band was not always complete until six hours had elapsed, but thereafter there was no loss of urobilinogen or urobilin for a long time, although this was not the case with the zinc acetate filtrates before the addition of Ehrlich's solution. The reading was made in the same way as with the urine and the total amount calculated for the volume of stool after grinding up with water, the dilution of the 25 c.c. by acid alcohol and zinc acetate being taken into account. Instead of using tap-water for dilution of the final extract, 60 per cent. alcohol was required to avoid the development of a precipitate."

Wilbur and Addis found that, while urobilin is a normal constituent of the urine, the amount is so small that ordinarily it can be demonstrated only by extracting large quantities of urine. A positive result, therefore, in twenty-four-hour specimens indicates an abnormal increase over the amounts usually present. In the stools of ten adults with supposedly normal haemoglobin metabolism they found that the average daily excretion of urobilin varied between

3307 and 8737 dilution values, with a general average of 6475. It is important, however, to note that, on account of the wide daily fluctuations, examinations of single twenty-four-hour specimens are of little value. So long as stools were kept from exposure to light there did not seem to be a very rapid loss of urobilin, and, as the desired figures represent the combined value of urobilinogen and urobilin, the amount of the former that changes to the latter while standing is immaterial. In making comparative studies, however, as for example before and after splenectomy, it is advisable to make examinations as nearly as possible after equal amounts of time have elapsed since the collection of the material. While Wilbur and Addis were not able to demonstrate urobilin in the blood-serum with certainty, they found their method was applicable to the study of the bile, but that widely-varying figures were obtained. Thus in sixteen diverse cases, unconnected with excessive blood destruction, 10 c.c. of bile, obtained post-mortem, yielded dilution values varying between 0 and 4500. As will be shown later, this finding constitutes a strong objection to the duodenal tube method of Schneider. In applying their method to various clinical conditions, Wilbur and Addis found very high average figures in the stools of cases associated with increased blood destruction. That this finding may be useful in diagnosis is shown by the fact that, whereas two cases of pernicious anæmia had average figures of 22,014 and 24,977 respectively, a case of secondary anæmia showed an average of only 2400. The value of this method in prognosis and in studying the results of treatment is shown in the following paragraphs.

By Charnas's method, Eppinger found that the normal adult excretes from 0.12 to 0.15 gm. of urobilin per day, and that these figures were not materially increased, or were even diminished in such secondary anaemias as those of carcinoma, Addison's disease, post-abortive anaemia, and chlorosis. In cases where there was excessive blood destruction, on the other hand, these figures were greatly increased. In pernicious anaemia, for instance, he found such figures as 0.24 gm., 0.58 gm., and 1.14 gms. per day, and in three cases of haemolytic jaundice the enormous increase of 1.75 gms., 2.59 gms., and 3.95 gms. per day. McKelvy and Rosenbloom²⁶² also found increased urobilin excretion in a case of haemolytic jaundice. In a case of pernicious anaemia submitted to splenectomy, Eppinger found that the increased urobilin excretion was diminished to mere traces after operation, and one of the cases of haemolytic jaundice, which excreted before operation 2.96 gms. to 3.95 gms. daily, after splenectomy excreted only 0.062 and 0.70 gm. daily.

These findings have been confirmed by Robertson,³⁷⁸ and also by work from this department (see page 202). Robertson emphasizes the fact that cases which had shown a high urobilin excretion before splenectomy, and in which after splenectomy the urobilin output exhibited only a transient reduction, or none at all, did not show as much improvement in other respects following the operation as did those cases in which the urobilin output was permanently reduced after splenectomy. The cases of pernicious anaemia and of congenital haemolytic jaundice studied in this department before and after splenectomy have been treated in detail in a previous chapter. In this connection,

however, it should be noted that splenectomy produced a definite improvement in the combined urobilinogen and urobilin excretion in both of these cases. In the case of pernicious anaemia before splenectomy the urobilin figure (by the Wilbur and Addis method) averaged 18,300 per day. Two weeks after splenectomy this figure averaged 16,000 per day, a diminution too slight to permit of significance being attached to it. Two months after splenectomy, however, at a time when the blood count showed a pronounced and most satisfactory improvement, the urobilin output had fallen to one-seventh of its former figure and had reached a low normal elimination. In the case of congenital haemolytic jaundice, urobilin was never found in the urine. In the stools, however, the dilution required for extinction of urobilinogen and urobilin absorption bands (Wilbur and Addis method), over two periods totalling ten days before splenectomy was 71,250; whereas for two periods totalling eight days after splenectomy the figures were only 7954.

Schneider³⁹⁶ has recently shown, by a method of his own devising, that a quantitative estimate may be made of the urobilin, urobilinogen, and bilirubin in the duodenal contents, obtained through a duodenal tube. He confirms previous work in finding all the above elements increased in pernicious anaemia and diminished or absent in other conditions simulating pernicious anaemia. He considers the pleochromia found to be an expression of the immediate haemolysis, urobilinocholia an expression of the heaped-up pigment in the portal system, and the high color-index of the blood in pernicious anaemia an expression of the overplus of haemoglobin-building material heaped up in the liver. Examination of a single specimen, as practised

in this method, however, even though the substance sought for may be in greater concentration, is so much less desirable than examination of the total collection of several days, as in the stool method, that it is doubtful whether this procedure will prove as useful as the Wilbur and Addis test, cumbersome and disagreeable as the latter may be. It has already been pointed out, also, that Wilbur and Addis found extremely wide variations in the urobilin content of bile, and that there is evidence to show that the daily output of urobilin varies greatly in a given individual. Although the stool method fails to take account of the urobilin reabsorbed by the intestine, this disadvantage is probably more than counterbalanced by weaknesses in the duodenal method, which ignores variations in the composition of the bile and estimation of the total quantity of bile secreted, and also is more liable to errors in the collection of material.

E. PROTEIN, URIC-ACID, AND IRON METABOLISM

Expression of the need for proper metabolic studies in the diseases considered in this book, the best methods for studying the same, and the results hitherto obtained, both experimentally and clinically, will be found in detail in Chapters VIII and X. In spite of the fact that discordant results are there recorded, both in clinical and experimental work, a few facts of value to the clinician may safely be deduced. In severe anaemias an increased elimination of uric acid and iron is found, and Umber's work demonstrates a pathologically excessive destruction of protein in some cases of Banti's disease. As all these conditions of metabolism improve after splenectomy, cases

showing such disturbance of metabolism should probably be considered especially suitable for splenectomy. A final decision on this point, however, cannot be reached until a larger number of metabolic studies are at hand, and for this reason it is desirable that complete studies of this kind should be made whenever conditions are favorable.

CHAPTER XIII

TREATMENT OF SPLENIC DISEASES BY METHODS OTHER THAN SPLENECTOMY

(1) BLOOD TRANSFUSION. (2) MEDICINAL AND HYGIENIC MEASURES. (3) SURGERY OTHER THAN SPLENECTOMY.

IN the treatment of certain phases or of individual cases of splenic disease, splenectomy must either be postponed or be considered as definitely contra-indicated. For instance, in very late stages of Banti's disease or in pernicious anaemia, in periods of exacerbation with extreme anaemia, or in the crises of deglobulization of the haemolytic jaundices recourse to splenectomy may be impossible. At the other extreme of the scale, some cases of familial jaundice, "more icteric than sick," are so little incommoded by the disease that splenectomy may be considered unnecessary. In these, as in other cases where the operation must be postponed, or even in relapses after splenectomy has been performed, certain other procedures have considerable value.

1. *Blood Transfusion*.—The most important of these is the method of multiple blood transfusions, which, on account of the simpler methods of technique now in use, both for detecting suitable donors and for the actual transfusion, has now come into widespread use.

Technique of Tests for Haemolysis and Agglutination.—Lindeman's²⁴⁹ method of testing the suitability of donors is as follows: "The red blood-cells of patient and donor are washed three times with normal saline; varying

quantities of patient's serum are placed in three separate small test-tubes. To each of these are added 0.25 c.c. of a 2 per cent. suspension of washed red blood-cells of the donor. The same is done with the donor's serum and the patient's cells. Controls are made of donor's serum and donor's cells—patient's serum and patient's cells. Controls are also made with donor's cells in normal salt solution and patient's cells in normal salt solution. The total volume in each tube is raised with normal saline to 0.5 c.c. of volume. The test-tubes are incubated in a water-bath for a period of two hours, and readings are made. They are then set in the ice-box over night and readings are made again the following morning. When a case is urgent, the ice-box test is eliminated. The ice-box test should be eliminated only when absolutely necessary by the extreme condition of the patient, where time is the important factor. When the amount of blood taken from the patient for tests is small, only 0.25 c.c. of serum is used, and controls of patient's serum are eliminated." A Wassermann test of the donor's blood should, whenever possible, be included. This and the subdivision of the donor's blood into the proper agglutination groups may readily be done, if the practice is followed of having in reserve a list of prospective donors ready to donate blood on demand.

In 146 cases in which tests by this method were personally supervised by Lindeman "not a single case of haemolysis and not a single death referable to the transfusion occurred. The necessity for careful performance of the test is shown by the fact that chills occurred in only thirteen instances (9 per cent.), whereas in nine cases in which the tests were not personally supervised chills occurred in five instances (55 per cent.)."

Minot²⁹² has shown that if one has on hand serum and corpuscles of subjects belonging to Groups II and III (of Moss's four groups), the group to which an adult patient belongs may be determined in twenty minutes. If then donors are available that have been previously catalogued according to groups, transfusion may be performed without danger.

The test is performed as follows: A suspension of red cells from the patient is "obtained by collecting one drop of blood in about 1 c.c. of a 1.5 per cent. of citrate solution in 0.9 per cent. salt solution." A drop of this suspension is mixed separately with a drop of each serum (which retains its agglutinating power for months), and allowed to stand fifteen or twenty minutes. The presence or absence of agglutination is then observed microscopically. Similarly a drop of the patient's serum is mixed separately with a drop of suspension of corpuscles known to belong to Groups II and III, and treated as above described. Minot also points out that haemolysis does not always occur *in vivo*, when donor and recipient belong to different iso-agglutination groups, because only about 20 per cent. of sera that are agglutinative are haemolytic. Haemolysis, however, never occurs without being preceded by or associated with agglutination. "Even when donor and patient belong to the same iso-agglutination group, however, there may occur, after transfusion, reactions of unknown nature, which are probably of not so severe or serious a nature as haemolysis."

While the technique of the various methods of transfusion now in use cannot be considered here, suffice it to say that, with proper precautions, untoward accidents may be practically eliminated and excellent symptomatic results

usually obtained. In all the chronic anaemias under consideration, not only is the degree of anaemia greatly lessened after transfusion, but in pernicious anaemia especially, a bone-marrow reaction, similar to the "blood crisis" following splenectomy, is apt to occur four to ten days after transfusion. Such a reaction "furnishes the most favorable time to do a splenectomy in those cases which have been transfused in preparation for the operation" (Vincent)⁴⁵⁴ and frequently initiates in pernicious anaemia a remission of from three to twelve months. It might then be asked: Why not continue to transfuse instead of having recourse to a major operation such as splenectomy? Unfortunately, (1) the benefit is probably of shorter duration than is the benefit conferred by splenectomy; (2) in some cases improvement becomes less and less after each transfusion, and (3) the increased blood destruction, as shown by urobilin excretion, is not lessened as it is by splenectomy.

In a study of 212 blood transfusions in 189 cases, Ottenberg and Libman³²⁵ discuss at length the various conditions in which transfusion is indicated. Although the best results were found in such conditions as simple hemorrhage, hemorrhagic diatheses, and acute poisoning, improvement was also noted in various infections, debilitated conditions, and in the chronic primary anaemias. In twenty-five transfused cases of pernicious anaemia, "fourteen underwent more or less prolonged remissions immediately following transfusion, and eleven showed little or no effect. Of these eleven, three were moribund at the time of transfusion and died within a few hours or days. The other eight, in spite of marked rise of haemoglobin and temporary symptomatic improvement, showed no interruption in the course of the disease, but continued to show blood destruc-

tion of about the same rate as before transfusion. Of the fourteen patients who showed progressive improvement following one or more transfusions, one had a remission lasting approximately three months, three had remissions lasting six or more months, three lasting a year or more, and three lasting over two years." In spite of the remissions that are known to occur spontaneously in pernicious anaemia, a record such as the above leaves but little doubt as to the value of blood transfusion in this condition. Formerly when the technique of whole blood transfusions was difficult single large doses were employed, but with the simpler methods now in vogue repeated transfusions of 400 to 600 c.c. are considered preferable in the chronic anaemias. Not only is the desired amount of blood easier to obtain and the discomfort to the donor lessened, but also the danger of hypertransfusion is avoided and the indications about as well met as when larger doses are used. As many as fourteen transfusions have been employed with benefit in chronic conditions (McClure²⁵⁹). It must be recognized that in some refractory cases the later transfusions in a series have done more good than the earlier ones; but it is usually true that if the procedure has failed in the first instance, it will probably continue to do so in the future, but is less apt to do so if a different donor is used. From a study of seven cases of pernicious anaemia which had been splenectomized and later transfused, Vogel and Downes gained the impression that the subsequent effects of transfusion were more marked and persisted longer in such cases than in those in which splenectomy had not been performed.

It would therefore seem that in pernicious anaemia, if signs of increased haemolysis are present, transfusions

should be employed until the patient is in the best condition to submit to splenectomy; and again after splenectomy, when the effect of the operation has passed away. If, on the other hand, the case is of the steadily progressive type, unsuitable for splenectomy, transfusions may be employed as a palliative measure, according to the state of patient's condition and purse. In the various "indirect methods" (syringe, Erlenmeyer flasks, citrated blood, etc.) the amount of blood transfused can be accurately measured, but even in the "direct" methods an approximate estimate of the amount of blood received may be obtained by accurately weighing the patient before and after transfusion.

2. Medicinal and Hygienic Measures.—Medicinal and hygienic measures to be employed in the treatment of the diseases under discussion are the usual remedies for anæmia, such as iron, arsenic (salvarsan), improved general hygiene, and an ample but simple, nutritious diet. Conflicting results have been reported from X-ray treatment of the spleen, but it is possible that properly graded doses may be of distinct value. It is almost superfluous to add that in cases of splenomegaly with anæmia, where a causative factor such as malaria, lues, uncinariasis, etc., is known to exist, appropriate medication is all-important. Up to a few years ago, before splenectomy and blood transfusions came into vogue in the treatment of chronic anæmia, various medicinal remedies were reported as of value. Organotherapy (spleen and bone-marrow feeding, Chauffard, Widal) and cholagogues (Chauffard, Cavazza⁶⁸), proved of but little help, and only with a long-continued course of high iron diet did Widal find any marked improvement. On account of the antihæmolytic properties of arsenic

(Gunn and Feltham¹⁶⁰), cholesterol (Chauffard and Grigaut,⁷⁴ Parisot and Heully³²⁸), and calcium chloride (Iscovesco¹⁹⁴), these drugs have been tried, in some cases with considerable improvement.

On the whole, however, it must be recognized that in the so-called primary anaemias medicinal treatment is at best palliative, and in most cases unsatisfactory, if relied upon for more than a short period.

3. *Surgery other than Splenectomy.*—A few surgical measures other than splenectomy have been employed in these conditions, but may be dismissed in a few words. Chauffard and Troisier,⁷⁵ Marchiafava and Nazari²⁷³ attempted to influence haemolytic jaundice by various surgical attempts on the bile-passages, but their failures are to-day only of historical interest. A peculiar operation, designed to diminish splenic function by squeezing the organ in new-formed connective tissue, is recommended by Schiassi,³⁹³ under the term "splenocleisis," for cases in which splenectomy could not be performed. The capsule of the organ is scarified, and wrapped with iodoform gauze, which is later gradually withdrawn. Destruction of the spleen by gradual cauterization, after fixation to the abdominal wall, has also been suggested, but the field for such a procedure would seem to be extremely limited and the chances of success not very great. In similar cases Troell⁴⁴¹ recommends, on the basis of experimental work similar to that done in this laboratory (page 121), ligation of the splenic arteries and veins. Lanz²³⁷ found ligation of the artery beneficial in three cases of wandering spleen, but Roblee³⁸⁰ states that out of six cases in which ligation was tried by Dr. Skel (discussion of Harris and Herzog's paper¹⁶⁹), four died. Although there is no theoretical or

experimental reason for such high mortality, this operation must still be considered to be in the experimental stage.

The most promising results from surgical measures other than splenectomy are to be found in the recent studies of Percy³⁴⁴ on the effects of removing chronic sources of infection in cases of pernicious anaemia in which the spleen is also removed. He found in pernicious anaemia not only that chronic inflammation may frequently occur in the gall-bladder, appendix, and other organs, as well as in and about the spleen itself, but also that the same strains of bacteria may frequently be cultivated both from the spleen and the other organ or organs involved. In the hope that a possible cause or contributing factor to pernicious anaemia may thus be eliminated, he therefore routinely removed gall-bladder, appendix, tonsils, or carious teeth, or as many as showed signs of chronic infection, either simultaneously with the spleen or as soon thereafter as was practicable. Even the energetic treatment of a complicating pyorrhœa has apparently reinforced the improvement caused by splenectomy. Of twenty-four cases treated in this way, twenty-one showed a marked postoperative improvement, and fourteen of these have still continued in their improved conditions over periods lasting from eight to thirty-one months. In view of these excellent results and as long as the cause of pernicious anaemia remains unknown, it therefore seems highly advisable to supplement splenectomy in this condition by the above procedures in any patient in whom such signs of chronic focal infection can be demonstrated.

CHAPTER XIV

TREATMENT: VALUE OF SPLENECTOMY AS A THERAPEUTIC PROCEDURE

SPLENECTOMY for rupture or severe injury of the spleen is one of the oldest abdominal operations about which we have definite knowledge.²²⁶ It was not until the advent of anaesthesia and the greater surgical skill of the nineteenth century, however, that it was found practicable to remove the chronically diseased organ and thus widen the field of applicability of the operation beyond that of surgical emergencies. Unfortunately, among the chronic diseases of the organ first attacked were the enlargements incident to cirrhosis of the liver and leukaemia. The unfavorable results in these two diseases cast discredit upon the operation; but, nevertheless, in 1908, Johnston²⁰² was able to collect 708 cases of total extirpation of the spleen with 194 deaths. From 1900 to 1908 there were 355 cases with 66 deaths. If the cases of leukaemia and trauma are subtracted, the list is reduced to 235 cases with 27 deaths, or a mortality of 11.5 per cent. Since 1908 our greater knowledge of the physiology and pathology of the spleen has resulted in a better selection of cases, so that now the total mortality has been somewhat further reduced (Laspeyres,²³⁸ Michelson,²⁹⁰ Mayo²⁸²).

In the past three years a more active study of the surgical treatment of certain so-called primary anaemias has led to the much more general use of splenectomy, and it is this application of splenectomy that attracts most attention at present.

CONTRA-INDICATIONS.—It is most important to know when splenectomy should not be done. We now know that in certain diseases removal of the enlarged spleen as a curative measure is contra-indicated. These include the various forms of leukæmia, also polycythaemia, and most cases of malaria, syphilis, and tuberculosis. In certain cases of cirrhosis of the liver (including the hypertrophic form) Eppinger has recently advocated splenectomy on account of the evidences of increased blood destruction in this disease when jaundice is a prominent feature; but in the ordinary atrophic forms in the absence of jaundice the desirability of splenectomy is questionable. Too much emphasis cannot be laid on the necessity of ruling out atypical forms of leukæmia—before the splenectomy is undertaken; but, on account of the great variety of aleukæmic conditions, this is often an extremely difficult task. In no case, however, should splenectomy be advised until the blood picture has been carefully studied over an extended period of time and the presence of leukæmia excluded so far as may be possible.

Anything pointing toward a hemorrhagic diathesis should also be given careful consideration. Its presence is, as a rule, sufficient to contra-indicate operation, although the repeated hemorrhages from varices or due to other mechanical causes, as in Banti's disease, are more indications for operation than otherwise.

In the severer anæmias definite signs of bone-marrow activity should also be forthcoming (nucleated or reticulated cells, Jolly bodies, etc.). If they cannot be provoked by appropriate drugs or by transfusion, it is probable that the marrow is relatively aplastic and splenectomy should not usually be attempted.

That the removal of the normal spleen is followed by a temporary anaemia has been shown both by clinical observation and animal experimentation; but this should not be considered a contra-indication to operation. The apparent paradox that, while removal of the normal spleen causes a temporary anaemia, removal of the spleen in certain blood diseases relieves the existing anaemia, has been commented upon in another section.

LEUKÆMIA.—On account of the almost invariably fatal outcome of splenectomy in early cases of leukæmia, for some decades the operation has been considered as definitely contra-indicated in this condition. Occasional cases have been reported, however, in which death did not follow splenectomy, and, in addition, the success that has attended this operation in recent years has inevitably tended to have the procedure applied to conditions which had previously been considered unsuitable. To help prevent the unwarranted inclusion of leukæmia in the scope of splenectomy, some evidence is here furnished as to the result hitherto obtained in this condition. In 1898, Vanverts⁴⁴⁷ collected twenty-nine cases of splenectomy for leukæmia, to which Fevrier¹¹³ adds two more. Of the thirty-one cases, only three survived the operation. Of these three cases there has been considerable doubt as to whether Franzolini's was really a case of leukæmia, and Banti did not hesitate to call it a case of Banti's disease. Burkhardt's case was apparently operated upon in an early stage (leucocyte count about 43,000). That the course of the malady was not influenced by the operation is shown by the fact that the patient died eight months later with the typical physical signs and blood picture of leukæmia. In the third case (Hartmann's) improvement lasted for three years, but

emaciation and gingival hemorrhages then became apparent. The cause of death in almost every unsuccessful case is hemorrhage, whether from the pedicle of the spleen, from torn adhesions between the spleen and diaphragm, viscera, or parietal wall, or from the laparotomy wound. In the single case that I have had opportunity to examine, an aleukæmic leukæmia, with a total leucocyte count ranging from 10,000 to 15,000, but with myeloid cells, the spleen was removed apparently without hemorrhage, but the abdominal wound showed no tendency to heal. Ventral hernia could not be avoided, and the patient died two weeks after operation from an acute generalized peritonitis.

In a few cases, where the great size of the spleen or a complication such as extreme mobility has been the prominent symptom, splenectomy has been practised as a palliative measure. Kuttner²³³ reported one such case of the myelogenous type that continued to improve while under observation, but he recognized that the progress of the disease had not in any way been influenced by the procedure in a curative sense. On account of the high postoperative mortality and of the evidence that the course of the disease is not affected by the operation, it is safe to say that splenectomy is definitely contra-indicated in the various forms of leukæmia. This is especially true in the acute form, while in the more chronic forms it should only be undertaken when the most urgent indications are present.

POLYCYTHÆMIA RUBRA.—On account of the fact that polycythæmia rubra (erythræmia, Vaquez's disease) is the only primary disorder of the red blood-cell system in which the cell count is increased, and that splenectomy has been attempted unsuccessfully in this condition, it seems advisable to consider it in some detail at this point, although it

cannot, of course, be included among the anaemias. First described by Vaquez⁴⁴⁸ in 1892, this peculiar syndrome was later brought more prominently before the medical profession by Osler³²⁴ in 1903, and its various features more exhaustively studied by Cominotti,⁸⁴ Hirschfeld,¹⁷⁸ Senator,⁴⁰³ Abeles,² and others. According to Lutembacher, whose monograph and bibliography present the most complete study of this disease, the salient symptoms are (1) a true erythrosis of skin and mucous membrane (rather than cyanosis, which may also be present), (2) dilatation and engorgement of the veins of the skin and retina, (3) gastro-intestinal disorders, albuminuria, vertigo, headache, somnolence, and lassitude (all due to visceral plethora), (4) pains resembling erythromelalgia, (5) polyglobulia (up to nine or eleven million erythrocytes per cubic millimetre), relative increase of polymorphonuclear leucocytes, with attempts at medullary reaction, (6) enlargement of the liver and especially of the spleen, though these features are not a necessary part of the syndrome. The resistance of the erythrocytes to various haemolytic agents has seldom been tested in polycythaemia. In most cases it has been found to be normal, although Guinon, Rist, and Simon¹⁵⁸ reported a case in which the resistance to hypotonic salt solutions was slightly increased. Pickard³⁵⁴ has very recently described a case of true polycythaemia in which haemolysis began at 0.48 per cent. NaCl and was complete at 0.30 per cent. NaCl (essentially normal limits). An increased resistance to a different kind of haemolytic agent, on the other hand, was shown by the fact that to antihuman haemolytic amboceptor the patient's cells showed no haemolysis when mixed with twice the amount of amboceptor necessary to haemolyze normal cor-

puscles. That this is not always the case in polycythaemia, however, is shown by the findings of Freund and Rexford,¹²⁹ in whose case the same test was performed and the resistance found to be normal.

The etiology is completely unknown, though it is probable that various toxic or infectious agents may provoke the syndrome, either by direct marrow stimulation or through excessive repair after a primary red blood-cell destruction. Thus Belonowsky was able to raise both haemoglobin and erythrocyte count by the frequently repeated injection of minute doses of haemolytic serum. In true cases it is possible to rule out all mechanical causes, such as occupation, hypertension, heart and lung disease, and adenopathy and other alterations in the blood should not be present. The pathogenesis of the disease is probably due to a true bone-marrow hyperplasia, as is shown by the fact that the over-active bone-marrow is but seldom forced to deliver immature or nucleated red blood-cells to the circulation. All the symptoms can be explained as a result of this plethora. The enlarged spleen is largely spodogenous; *i.e.*, compensatory attempt to provide for excessive blood destruction, with resultant increased macrophagic action and congestion.

On *a priori* grounds, therefore, it will readily be seen why splenectomy should not be undertaken in this disease. If it is correct that the primary trouble is in the hyperplastic bone-marrow, the overactive spleen should be looked upon as the chief agent to keep the plethora within limits compatible with life. A logical radical treatment would be the obliteration of the marrow of one or more long bones, or ligation of nutrient arteries, just as partial excision of the thyroid causes improvement in exophthalmic goitre.

Such procedure has not to my knowledge been attempted, and it is doubtful if a sufficient effect could be produced to make it of clinical value. As a matter of fact, splenectomy has been but rarely attempted, although Lutembacher states that "it has been followed in several cases by a rapidly fatal termination from suppuration or hemorrhage, and in those cases that survived it has caused an evident augmentation in the polyglobulia." A confirmation of the protective action of the spleen in this condition is afforded by the increase in polycythæmia in cases where enlarged spleen has been reduced in size by X-ray treatment. In some cases, on the contrary (perhaps where the X-ray dosage to the spleen has been irritative rather than destructive), this form of treatment has proved beneficial. Destructive X-ray applications to the long bones have not proved of value. Large venesections, repeated at rare intervals and followed by salt-solution injection, have also been of use in some cases, but the consequent improvement is always evanescent and, on the whole, this treatment must be considered unsatisfactory.

DISEASES IN WHICH SPLENECTOMY MAY OCCASIONALLY BE INDICATED

CIRRHOSIS OF THE LIVER.—Mention has already been made of the fact that Eppinger had presented an experimental basis for extending splenectomy to cases of cirrhosis in which jaundice was a chronic and prominent symptom. On account of the enlargement of the spleen that frequently is found at an early stage of cirrhosis of the liver, Eppinger¹⁰⁴ was led to search for evidences of increased blood destruction in this connection also. This was forthcoming, when jaundice was present, not only in the exist-

ence of increased anæmia, but also in the increased amount of urobilin found in the stool and the high iodine number of the blood. With less justification, but on account of the analogy that exists between the changes in the liver and spleen in portal cirrhosis and in Banti's disease, other authors have attempted splenectomy in portal cirrhosis even in the absence of jaundice or other signs of excessive blood destruction. Thus Jullien,²⁰⁵ in 1911, reports seven cases treated in this way, of which two died as a result of the operation, while the others showed considerable improvement. This even included such important changes as the long-continued disappearance of a chronic ascites, and of the superficial evidences of collateral circulation. Kidd²¹¹ believes that splenectomy should be tried in all cases of cirrhosis showing enlargement of the spleen, and W. J. Mayo²⁸¹ has also found splenectomy advisable in cirrhosis of the liver. In addition to one case of Hanot's cirrhosis in which the spleen was removed with "undoubted benefit and possible cure," he has removed the greatly-enlarged spleens from four patients suffering from portal cirrhosis. Although it was too early to know whether or not the end results had justified the operation, three of the four patients showed marked improvement with disappearance of the ascites and anæmia. Although the evidence is still meagre on this point, and the evidence at hand insufficient to determine what types of cirrhosis are included under this term by surgeons, the results are favorable enough to entitle the procedure to further consideration. In estimating their value, however, it should be remembered that the differential diagnosis of portal cirrhosis from Hanot's cirrhosis and from Banti's disease and other similar conditions is often difficult or impossible

to make, and it should also be borne in mind that there is removed with the spleen a reservoir which to some authorities is of great importance in accommodating the blood which accumulates behind the obstacle of the cirrhotic liver. Whether or not this very accumulation of blood in the spleen is a factor which may lead to increased blood destruction is a point for the future to decide.

MALARIA AND SYPHILIS.—The extreme size attained by the spleen in chronic malaria and the frequency of malaria in tropical regions early turned the attention of French and Italian surgeons toward the advisability of splenectomy in this condition. In spite of almost uniformly fatal results in the early cases, surgeons have persisted in their attempts, on account of the many distressing symptoms (dyspnœa, cyanosis, vomiting, dysuria, dysmenorrhœa, abdominal pain, etc.) that the tremendously enlarged spleen may cause. The operation has been, however, attended with unusual difficulties on account of the friability of the organ and vessels and the great number and density of the adhesions in most cases. This not only promotes the liability to severe hemorrhages, but, on account of the length of time required for the operation, greatly increases the shock of the operation. Of the twelve deaths in the series of forty-seven cases collected by Olgiati,³²¹ ten were due to these causes, the other two to peritonitis. Twenty of the thirty-five cases that recovered, on the other hand, had ectopic spleens, which, therefore, did not present adhesions to the surrounding organs. As it is precisely this type of spleen that is especially liable to the further complication of torsion of the pedicle and rupture, exploratory laparotomy would be indicated if the enlarged spleen had failed to respond to medicinal treatment and was causing

distressing symptoms. It should, however, be recognized that splenectomy was undertaken for the relief of such symptoms and not as a curative measure. In Roumania splenectomy has been practised in resistant cases of chronic malaria with considerable improvement in many cases, but with the high operative mortality of over twenty per cent. (Racoviceanu³⁶⁹). As all these figures, however, date from more than fifteen years ago, the better technique of the surgery of to-day may eliminate shock and hemorrhage to such a degree that the mortality in malaria may prove to be no higher than in the other diseases.

In some cases of long-standing syphilis, also, when the enlarged spleen has proved resistant to specific treatment, it may be advisable to remove the organ that has become the chief cause of the patient's disability. Thus W. J. Mayo²⁸² has removed the greatly-enlarged spleen from three patients suffering from chronic syphilis and marked anæmia. "In one of these specific treatment had been carried out for two years, in another for six months, without satisfactory improvement in the general condition of the anæmia. Following splenectomy, there was marvellous improvement of the anæmia in all of them."

The ultimate results of splenectomy in such cases will be awaited with great interest. It may prove that occasionally the enlarged spleen, at first protective, eventually assumes a pernicious activity, or that, like the central nervous system in some chronic infections, it may become a secluded nidus of infection which cannot be reached by ordinary medical treatment. In either of such contingencies its removal, therefore, may become desirable. Nevertheless, for the present at least, enlarged spleens should only be routinely removed in those cases where the

indications have been proved to be favorable or else where sudden emergencies require a greater latitude in the employment of this form of treatment. The widespread removal of spleens without accurate diagnosis or regard to the suitable indications will undoubtedly lead not only to many disastrous results but also to the indefinite obscuration of the proper field for this important operation.

TABLE LXIV
COLLECTED REPORTS OF RESULTS OF SPLENECTOMY IN VARIOUS DISEASES OF THE BLOOD

Disease	Author	Number of cases	Recovered	Died	Per cent. mortality
Gaucher's.....	Erdman and Moorhead ¹⁰⁶	10	8	2	20
Banti's.....	Krumbhaar	183	155	28	15.4
Hæmolytic icterus acquired	Elliott and Kanavel ¹⁰²	16	15	1	6.2
Hæmolytic icterus, congenital and familial	Elliott and Kanavel ¹⁰²	23	22	1	4.3
Hæmolytic icterus, unclassified	Elliott and Kanavel ¹⁰²	9	9	0	0
v. Jaksch's.....	Stillman ⁴¹⁷	6	6	0	0
Pernicious anæmia....	Krumbhaar	153	123	30	19.6

DISEASES IN WHICH SPLENECTOMY IS INDICATED

In certain types of chronic anæmia, splenectomy has met with considerable success; as in Banti's disease, Gaucher's disease, the congenital and acquired forms of hæmolytic jaundice, and, to a lesser extent, pernicious anæmia. The cause of the improvement or cure that follows splenectomy in these conditions is but incompletely understood, and is probably different in the various diseases mentioned. Some of the factors at work have been considered elsewhere in this book.

BANTI'S DISEASE.—In Banti's disease it is important that the operation should be undertaken before the disease

has progressed beyond the first stage. Splenectomy in the first stage is not only accompanied by a lower mortality, but in the great majority of cases has caused great and lasting improvement in symptoms, often amounting to a complete cure. When the third stage is reached, with permanent changes in the liver and circulatory system, not only is the operation more dangerous, but the chances of improvement are greatly lessened. As a result of the disease process, the spleen has by this time become largely fibrotic, and its removal could hardly be expected to be attended with marked beneficial effect.

In 1907 Torrance collected thirty-six cases of Banti's disease, of which nine died as a result of the operation, a mortality of 25 per cent. A year later Johnston added twenty-five cases with only three deaths, making an operative mortality of reported cases up to 1908 of 19.7 per cent. By a far from exhaustive survey of the literature since 1908 we have added 122 cases with sixteen deaths that were not in Torrance's or Johnston's lists, making a total of 183 cases with twenty-eight deaths—a mortality of 15.4 per cent. If the cases since 1910 are considered, however, there are found 76 cases with eight deaths, a mortality of only 11.1 per cent. Although allowance must be made for the more liberal publication of favorable results, this last percentage is the same as that for the largest collection from any one clinic (Mayo) and is probably very close to the present operative mortality for this condition. With proper selection of cases and technique, Mayo believes that this mortality percentage can still be cut in half. On account of the early reporting of most cases it is impossible to get an adequate idea of the ultimate outcome of cases surviving operation. Occasional reports can be

found in which a marked postoperative improvement was followed in one or two years by subsequent relapse or death (Roberts,³⁷⁷ Lett,²⁴⁶ Kidd,²¹¹ Giffin¹⁴²). Nevertheless, the prevalent opinion is probably correct that the improvement that follows splenectomy in early Banti's disease in the great majority of cases either amounts practically to a complete cure or persists without relapse for many years. Even in the third stage, splenectomy may be of value: thus of the twenty cases of this series that were operated upon in the third stage, ascitic or cirrhotic stage, five succumbed to the operation and at least three more were not materially helped and died within a few years. While these figures are less encouraging than those for the earlier stages, they by no means should be taken as proof that the operation is contra-indicated in this stage. As early as 1906, Jaffé¹⁹⁷ showed that, even with marked ascites and advanced cirrhosis, splenectomy, if combined with the Talma operation, may be attended with very striking improvement.

A patient, then, with the symptoms of Banti's disease, particularly if in the early stages of the disease, should be considered a proper subject for splenectomy, but the most favorable time for operation should be selected. Before undertaking the operation all other possible causes for such a syndrome (*e.g.*, aleukæmia, leukæmia, tuberculosis, malaria, syphilis, etc.) should be ruled out so far as possible by a complete but not unnecessarily prolonged investigation as to the cause of the disease, through frequently repeated blood examination. In the third or ascitic stage a combination of splenectomy with the Talma operation is advisable.

GAUCHER'S DISEASE.—On account of the rarity of this

condition and the difficulty of diagnosis without the aid of histological examination, not many cases are available for study. In 1914, Erdmann and Moorhead¹⁰⁶ collected ten cases of large-celled splenomegaly (Gaucher's disease) in which the spleen had been removed, and of these, two died, both within twenty-four hours of operation. While this probably represents too high a mortality, the improvement which followed in the other eight cases cannot always be taken as indicative of eventual cure, for the disease is known to exist independently in the bone-marrow and lymph-nodes. It would therefore seem wiser to restrict splenectomy in this disease to those cases that are unusually handicapped by the results of the disease, but are still good surgical risks, and in such cases to limit the prognosis to improvement and not to promise complete cure.

HÆMOLYTIC JAUNDICE.—The field in which splenectomy has been practised with the greatest success is undoubtedly that of hæmolytic jaundice. Both in the acquired form (Hayem-Widal) and the congenital or familial type (Chauffard-Minkowski), marked improvement and frequently complete cure have resulted from removal of the spleen. In fact, the success obtained in this type of case, where the chief vitium is that of increased blood destruction, has been a powerful incentive toward extending the operation of splenectomy to the wider range of allied diseases discussed in this chapter. Splenectomy was first tried in this condition by Vaquez and Giroux. As their case, however, died two days after operation, Chauffard's dictum that hæmolytic jaundice constituted a "*Noli me tangere*" for the surgeon prevailed for several years. In 1911, however, splenectomy was again tried in hæmolytic

jaundice with a very different result from that in Vaquez and Giroux's case. In that year Micheli removed the spleen from a case of the acquired type with the most striking improvement: the blood count, which had been between 980,000 and 2,600,000, quickly rose to almost normal, the acholuric jaundice and urobilinuria disappeared, the fragility of the red cells was lessened, and the patient within a few months was apparently cured. Similar beneficial results were obtained by Banti in his two cases of hæmolytic splenomegaly, which we have taken to be identical with the acquired form of hæmolytic jaundice. Success was obtained in other early operations (Kahn, Roth), and the procedure would undoubtedly have been in more frequent and intelligent use, in this country as well as Europe, if the unfortunate grouping of several clinical entities under the cloak of "Splenic anaemia" had not clouded the worth of the procedure. In 1915, Elliott and Kanavel¹⁰² were able to collect forty-eight cases of hæmolytic jaundice (sixteen acquired, twenty-three familial, and nine unclassified) that had been treated in this way. Of the forty-eight cases, only two died—one shortly after operation, the other from sepsis, six weeks after operation. The other forty-six are reported as "cured," this result being based upon the disappearance of jaundice and exacerbations, and decrease of the anaemia and the urobilin excretion. The effect on the resistance of the red cells was not constant; in some instances the resistance returned almost to normal, but in most cases the red cells remained almost as fragile as before operation. In spite of these brilliant results, however, it must be remembered that the primary cause of the disease is unknown and is probably not in the spleen. The case

reported by Whipham⁴⁶⁷ emphasizes this point; although splenectomy was followed by great clinical improvement, return of the red blood-cell count to a level above normal (polyglobulia) and a reduction of their fragility to a normal level, nevertheless, three months after operation, jaundice and extreme anæmia returned and the child died in a "crisis of deglobulization." "Whatever the *causa causorum*, it must be regarded as established that it is through the instrumentality of the spleen that pathologic haemolysis is wrought."

v. JAKSCH'S DISEASE.—The results of splenectomy in v. Jaksch's disease are too meagre to be of value. Only six cases have so far been reported (Stillmann^{417a}), and, although all of these were improved by the operation, it must be admitted that paediatricians claim even greater improvement or even cure after long-continued medical treatment of this condition. It should be noted that the first case reported by Stillmann exhibits certain features more compatible with the diagnosis of haemolytic jaundice than of v. Jaksch's disease (decreased resistance of erythrocytes, great increase in reticulated erythrocytes, and a practically normal leucocyte count).

PERNICIOUS ANÆMIA.—The most important disease, from the point of view of its greater frequency and greater severity, to which splenectomy has been applied is pernicious anæmia. The striking improvement that has been shown to follow removal of the spleen in such diseases as haemolytic jaundice and Banti's disease naturally led to an extension of this clinical procedure to allied conditions. In 1913 three investigators—Eppinger,¹⁰⁴ Decastello,⁹² and Klemperer,²¹⁶—working independently, tried splenectomy as a therapeutic measure in pernicious anæmia. It

is interesting that Eppinger was led to adopt this procedure by observing after splenectomy a diminished output of urobilin and other evidences of decreased haemolysis. Decastello, on the other hand, had noted the improvement that followed splenectomy in the related conditions, haemolytic jaundice and Banti's disease; whereas Klemperer was influenced by the clinical observation that splenectomy for such conditions as rupture of the spleen was in some instances eventually followed by polycythaemia.

Such marked improvement was noted in these earlier cases that the procedure was quickly and widely repeated, chiefly in Germany and in this country, so that a fairly large group has already become available for study. More prolonged observation, however, has shown a considerable mortality from the operation and postoperative complications, and, moreover, that very few of the patients continue steadily to improve; in almost all the characteristic blood picture of pernicious anaemia remained, and not a few died from relapses of the disease in the first year or two after operation. The object of this section is to show by a correlation of the published reports, with later information obtained by personal communication, just how valuable splenectomy has thus far proved to be in pernicious anaemia.^{230a}

Although it has been impossible to get additional reports from some of the German authors, and of some patients who have been lost sight of, nevertheless the results obtained in the last two and one-half years are sufficiently concordant to give evidence of some value. In drawing conclusions from any such review, however, it must be remembered that certain difficulties are unavoidable. For instance, the dividing line between pernicious anaemia

and some of the other primary blood diseases is necessarily such a shadowy one that the possibility of an incorrect diagnosis must always be borne in mind. Then, too, the concept of pernicious anaemia varies so much with different authorities that cases included as such by one author might easily be rejected by another. Thus one of the earlier cases of splenectomy that was followed by marked and long-continued improvement, when subjected to critical analysis, seems to be rather a case of acquired haemolytic jaundice than of true pernicious anaemia. In another case reported as pernicious anaemia the diagnosis was later changed to haemolytic jaundice on account of the subsequent appearance of acholuric jaundice with diminished resistance of the erythrocytes. As such variations in diagnosis are apt to include less serious diseases under the head of "pernicious anaemia," or diseases in which splenectomy is already known to be of benefit, the present statistics will be correspondingly favored by such inclusions. In applying the present figures to prognosis, however, it is fair to offset the inclusion of such cases with the results that must inevitably follow the better selection of cases and better preparation for operation. A third consideration is that the term "pernicious anaemia" may later be found to include more than one clinical entity. (Compare the great variations that occur in the size of the spleen, in the bone-marrow reactions, in the evidences of haemolysis, and in the duration of the disease.) If this were found to be true, it might well be that some of the apparently discordant results that have been observed after splenectomy are due to the fact that the operation was of value in one or more types and contra-indicated in the others. The results of these studies are included in Table LXV.

SPLENECTOMY TREATMENT

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RESULTS OF SPLENECTOMY IN PERNICIOUS ANEMIA

Observers	References	Number of cases	Postoperative results			Total deaths
			Deaths	No im- provement	Improved	
Decastello, A. von ⁴²	Deutsch. med. Wochenschr., 1914, xli, 639; personal communication to the author.	13	4	1	8	7
Eppinger, H. ¹⁰⁴	Berl. klin. Wochenschr., 1913, xxix, 2409; personal communication to the author.	7	0	0	7	?
Fahrnius, M. B. ¹⁰⁵	Förhandl. d. deutsch. Gesellsch. f. Chir., 1915, viii, 249; personal communication to the author.	3	1	0	2	2
Fenckel, M. ¹⁰⁶	Förhandl. d. deutsch. Gesellsch. f. Chir., 1914, xiii, 233; personal communication to the author.	2	0	2	0	1
Förcken, L. ¹⁰⁷	Förhandl. d. deutsch. Gesellsch. f. Chir., 1914, xiii, 231; personal communication to the author.	2	1	0	1	1
Frangenheim, M. ¹⁰⁸	Sitz. d. allg. Aerzt. Ver. z. Köln, München, med. Wochenschr., 1914, Ixi, 1760; personal communication to the author.	2	1	1	0	1
Giffen, H. Z. ¹⁰⁹	Personal communication to the author.	31	3	6	22	7
Guleke, O. ¹¹⁰	Verhandl. d. deutsch. Gesellsch. f. Chir., 1914, xlii, 227; Med. Klin., 1914, x, 1544; personal communication to the author.	2	2	0	2	2
Hansing, W. ¹¹¹	Cor. Bl. f. schweiz. Aerzte, 1914, xiv, 1171; personal communication to the author.	2	1	0	1	2
Hössli, H. ¹¹²	The Journal A. M. A., July 17, 1915, p. 216; personal communication to the author.	14	4	2	8	5
Klemeter, G. ¹¹³	The Journal A. M. A., March 6, 1915, p. 796; personal communication to the author.	2	1	4	10	3
Lee, R. ¹¹⁴	Personal communication to the author.	2	1	1	0	1
LongCOPE, W. T. ¹¹⁵	Surg., Gynec. and Obst., 1915, xxi, 360; personal communication to the author.	19	2	2	15	4
Percy, N. W. ¹¹⁶	The Journal A. M. A., March 6, 1915, p. 796; personal communication to the author.	2	0	0	2	1
Roblee, W. W. ¹¹⁷	Berl. klin. Wochenschr., 1914, II, 669; personal communication to the author.	2	0	0	2	1
Roemer, W. S. ¹¹⁸	Tr. Assn. Am. Phys., 1914, xxix, 489; personal communication to the author.	2	0	0	2	1
Thayer, W. S. ¹¹⁹	Personal communication to the author.	7	3	1	4	3
Vogel, C. ¹²⁰	Personal communication to the author.	3	0	0	3	0
Jacobi, N. von ¹²¹	Wien. klin. Wochenschr., 1914, xvii, 1536; personal communication to the author.	21	4	4	13	9
*Single Cases.....						
Total.....		153	30	24	99	53

*The single cases were reported by various authors as follows:

⁴² Anschütz, Verhandl. d. deutsch. Gesellsch. f. Chir., 1914, xliii, 232.¹⁰⁴ Baldwin, J. F.: Med. Rec., New York, 1915, lxxvii, 230.¹⁰⁵ Brill, N. E.: Tr. Assn. Am. Phys., 1915, xxi, 547.¹⁰⁶ Burger, L., and Ottenberg, R.: Med. Rec., New York, 1914, lxxxvi, 860; later report by personal communication to the author.¹⁰⁷ Coleman, W.: Tr. Assn. Am. Phys., 1914, xxi, 470.¹⁰⁸ Dahl, R.: Ryterie, 1914, xxvi, 471; later report by personal communication to the author.¹⁰⁹ Harpole, W. S., and Fox, C. W. N.: Surg., Gynec. and Obst., 1914, xxiv, 243; later report by personal communication to the author.¹¹⁰ Huber, O.: Berl. klin. Wochenschr., 1913, I, 2179; later report by personal communication to the author.¹¹¹ Port, F.: Berl. klin. Wochenschr., 1914, II, 546.¹¹² Rodman, J. S.: Personal communication to the author.¹¹³ Stewart, F. T.: Personal communication to author.¹¹⁴ Talley, J. E., and Johnson, J. H.: Personal communication to the author.¹¹⁵ Turk, W.: Deutsch. med. Wochenschr., 1914, XI, 371.

Analysis of Results.—It will be seen that of the 153 individuals whose spleens were removed, thirty died within six weeks, presumably from the effects of the operation, a mortality of 19.6 per cent. Of the remaining 123 patients, all but twenty-four showed a distinct improvement, both in general condition and in blood picture. Of the twenty-four individuals that survived the operation but failed to improve, a few were obviously harmed by it. To this group belongs Pappenheim's case, splenectomized at a favorable time, when the patient was in the stage of a remission. The condition, nevertheless, was aggravated by the operation: the blood showed signs of increased destruction and a serious relapse began. The improvement noted in the majority of cases lasted varying periods. Thus at the end of six months, of fifty-three patients who had survived operation for more than six weeks and were still under observation, forty-four had still continued to improve and none had died, but nine had already relapsed.

TABLE LXVI
LATE RESULTS AFTER SPLENECTOMY IN PERNICIOUS ANÆMIA

	After one year	After two years
Number cases known.....	27	6
Still improved.....	11	3
Relapsed.....	7	2
Died subsequently.....	9	1

At the end of the first year after operation there remained twenty-seven patients who were still under observation (see Table LXVI). Of these, Decastello's series is the most important, not only because he and Eppinger were the first to try this procedure and therefore the cases could be followed for a longer time, but because the early

successful results have been greatly modified by time. Of six patients at the time of his publication, several months after operation, four showed such great improvement that, except for the microscopic appearance of the blood, they might have almost been considered cured. Two years later, however, two were dead and one was in as poor condition as before operation. Of the other two important early series, Eppinger's and Klemperer's, it has been impossible to get additional information. The figures for the whole group of twenty-seven cases, however, show that the initial improvement has been maintained in less than half of the cases.

A small but interesting group is formed by six individuals (see Table LXVI) that have been known to have lived two years or more after operation (Decastello [two], Giffin, Harpole, Huber, Thayer). Of these, Giffin's patient had had the disease for two and one-half years; the anaemia was not extreme at the time of the operation, and the spleen was much enlarged (1640 gm.). Improved by the operation, he died three years later from pneumonia. In Decastello's two cases the disease had existed for less than a year; the anaemia was severe and the spleen but slightly increased in size. These patients improved after operation both clinically and as to the blood picture; but, whereas one in a subsequent report was in poor condition, the other was without symptoms, although the blood picture was still that of pernicious anaemia. Harpole's patient was known to have had pernicious anaemia for two years, and at the time of operation the anaemia was moderately severe and the spleen twice the normal size. After splenectomy there occurred immediately an active bone-marrow reaction with marked clinical improvement. The patient has

continued in fair health, with only a slight anæmia, but with persistence of spinal-cord symptoms. Huber's patient, who was considered moribund at the time of operation, improved rapidly for seven weeks, relapsed, and later underwent a spontaneous remission. After two and one-half years she was still in good condition and able to do her housework, but still anæmic. Thayer's patient, having had the disease one year, improved after splenectomy, although there was no bone-marrow reaction. After eighteen months the patient relapsed to the same condition as before operation and was last reported in poor condition.

Estimation of the value of such a procedure as splenectomy in pernicious anæmia must take into consideration not only the actual results obtained, but a comparison, so far as is possible, with the probable results if operation had not been undertaken. Thus, whereas we have seen that splenectomy caused a quick and marked improvement in 64 per cent. of all patients, natural remissions occurred at one time or another in over 80 per cent. of the patients of Cabot's⁶⁵ series treated by the older conservative methods. One cannot maintain from this that perhaps the improvement after splenectomy was only a coincidental remission, because the onset of improvement was too closely and constantly related to the postoperative period; but it does offer some basis for the contention that other methods of treatment may yield results as striking as those following splenectomy. However, from the aspect of duration of the disease the evidence is more in favor of the splenectomized series. In Cabot's series, almost half died in the first year of the disease, and of the remainder, one-third died in the next year (compare Table LXVII). As the duration of the disease in the splenectomized series had already

averaged one and one-half years before operation, they should be more properly compared with the remainder of Cabot's group. By the end of the first year conditions in the splenectomized group were as follows: Of thirty-three patients surviving the operation, twenty-four were still improved, three had failed to show improvement or had relapsed to their pre-operative condition, and six had died. If postoperative deaths, however, are included, only about half of those whose fate was known were still alive at the end of the first year. From both these points of view, therefore, there are no clear indications as to the value of splenectomy.

TABLE LXVII

RESULTS ACCORDING TO DURATION OF DISEASE, BASED ON NINETY-FIVE CASES

Duration	Number of cases	Postoperative results			Subse- quently died
		Deaths	Not im- proved	Improved	
Under 6 months.....	17	4	..	13	..
Six months to 1 year.....	26	4	3	19	3
One to 2 years.....	36	3	6	27	8
Over 2 years.....	16	6	1	9	4

The changes in the blood picture after splenectomy are striking and fairly constant. Forty-seven cases are stated to have had a distinct postoperative blood crisis (appearance of normoblasts, megaloblasts, reticulated erythrocytes, Jolly bodies, etc., in larger quantities); and, as statements that the blood crisis failed to appear are very rare, it is fair to assume that such a phenomenon is at least a frequent occurrence. In most of the patients who recovered the stimulation forms soon grew fewer in number and, coincident with the signs of general improvement, the haemoglobin and red-cell count rose. The color-index, how-

ever, usually remained high. As might be expected, also, the nucleated forms became fewer or disappeared; and yet statements are almost unanimous that the microscopic features of the blood (tendency to macrocytosis, poikilocytosis, etc.), even in many cases that showed almost normal counts, remained suggestive of pernicious anæmia. Those patients who died within the first six weeks after operation showed either very slight improvement in the blood picture or an actual deterioration; whereas, death occurring after that period was in many cases preceded by a distinct improvement in the blood picture, with the usual signs of a remission.

Those few patients who up to the present time have continued well after operation must also be taken into consideration. By the older methods of treatment, Cabot, in the first edition of "Modern Medicine," stated that six out of 1200 cases after six years of health might be said to have been cured. In the second edition this number was reduced to three. May it not develop that a larger percentage of such "cures" will follow splenectomy, even though the blood picture does not return absolutely to normal? Another point brought out by study of the blood picture at the time of operation is that if the operation is undertaken before the blood has reached an extreme degree of deterioration, not only is the operative risk lessened, but the improvement is greater and more lasting. In any case it is wise to precede it with one or more transfusions.

The average age of the patients at the time of operation (see Table LXVIII) was forty-five; the average duration of the disease at that time, 1.6 years. If the results are analyzed in groups subdivided according to age, it appears (as one would expect) that less favorable results are ob-

tained in patients in the sixth and seventh decades. A similar arrangement on the basis of duration of the disease shows that the best results are obtained (after the operation has been successfully passed) in those patients who have

TABLE LXVIII

RESULTS ACCORDING TO AGE OF PATIENT, BASED ON ONE HUNDRED AND TWENTY-ONE CASES

Age, decade	Number cases	Postoperative results			Subse- quently died
		Deaths	Not improved	Improved	
3d.....	8	2	1	5	1
4th.....	23	4	2	17	2
5th.....	43	5	2	36	8
6th.....	28	4	5	19	6
7th.....	9	5	2	2	1

had the disease for less than one year. Sex has not been found to exert any influence on the results.

When arranged according to the degree of anaemia at the time of operation, one fact is patent: that the operative risk is much greater in those cases in which haemoglobin is below 20 (see Table LXIX). This is, of course, for

TABLE LXIX

RESULTS ACCORDING TO PREOPERATIVE DEGREE OF ANAEMIA, BASED ON ONE HUNDRED AND FOURTEEN CASES

Haemoglobin	Number of cases	Postoperative results			Subse- quently died
		Deaths	Not im- proved	Improved	
Below 20.....	9	7	1	1	..
Below 30.....	29	6	2	21	3
Below 40.....	33	2	2	29	7
Above 40	43	7	7	29	5

this disease, a lower level even than that of 1,000,000 erythrocytes.

Except for this point, on account of the great fluctuations in blood counts incident to the disease and following

transfusions, it is difficult to estimate whether or not the previous condition of the blood has any marked influence on the result obtained. The impression is gained, however, that the best results follow splenectomy in those cases that are not extremely anæmic at the time of operation and that have shown considerable fluctuations in the blood picture.

Information as to the size of the spleen was secured in eighty-nine cases (see Table LXX). In twenty-eight cases (31 per cent.) the spleen was either small or approxi-

TABLE LXX
RESULTS ACCORDING TO SIZE OF SPLEEN, BASED ON EIGHTY-NINE CASES

Size of spleen	Number of cases	Postoperative results			Subse- quently died
		Deaths	Not im- proved	Improved	
Normal or diminished.....	28	9	5	14	2
Slightly enlarged.....	41		4	32	9
Considerably enlarged.....	20	1	1	18	3

mately normal in size. In forty-one cases (46 per cent.) the spleen was slightly enlarged (between 250 and 500 gm.) ; and in twenty cases (23 per cent.) it was considerably enlarged. In other words, although seldom palpable before operation, it was distinctly enlarged in over two-thirds of the cases. If the results of splenectomy are subdivided according to the size of the spleen, it will be seen that better postoperative results were obtained in the cases with enlarged spleens. The third group in this arrangement, however, is the only one that could be said to have fared better than another as regards the ultimate outcome of the disease.

THE EFFECT PRODUCED BY SPLENECTOMY.—Whatever the cause of the distinct improvement after splenectomy in those patients who survive the operation, it is obvious

that it is not due to the removal of the sole cause of the disease. If Eppinger's theory of thickened arteriole walls in the spleen, with consequent damming back and destruction of red cells in the splenic pulp, were correct, removal of the spleen should indeed cure the disease; but our studies show that this is not the case. On Eppinger's theory, also, the characteristic remissions of pernicious anaemia would be difficult to explain.

The postoperative blood crisis discussed above, and the subsequent improvement in the blood picture, decrease in urobilin, etc., indicate both that a stimulus has been applied to the bone-marrow and that a source of blood destruction has been removed. Lee's findings of an increased number of platelets after splenectomy would also support the bone-marrow stimulation theory. Hypotheses to explain these phenomena have been unsatisfactory and the relevant experimental evidence often contradictory. Klemperer believes that the bone-marrow activity is induced by removal with the spleen of an inhibiting hormone, but from experiments in our laboratory we have found³⁴⁰ (in normal animals, to be sure) not only that this bone-marrow activity does not occur until after several months have elapsed, but also that fresh splenic extract stimulates²²⁸ instead of inhibiting the bone-marrow. The cause for the blood crisis, therefore, must probably be sought elsewhere: perhaps, as has been suggested, in bone-marrow stimulation from metabolic products or from abnormal constituents of the erythrocytes that are allowed to remain in the blood by the removal of the spleen (Decastello). It is also difficult to prove that the lessened haemolysis after splenectomy, as shown by decreased output of urobilin, is actually due to

the absence of the spleen. Neither normal spleens nor those removed at operation in cases of blood disease (Coleman, Stewart, Robertson) can be shown to possess demonstrable haemolytic activity, and studies of the blood entering and leaving the spleen have also failed to throw light on the supposed haemolytic function of this organ (Krumbhaar and Musser ²²⁸).

Another factor to be considered is changes in the red blood-cells themselves. In one case that I had an opportunity to study (Stewart's), the resistance of the erythrocytes was distinctly increased after splenectomy, so that this might constitute one of the factors of improvement after operation. A similar increase in the resistance of the erythrocytes we have found to be the rule ²⁰⁹ after the removal of the spleen in normal animals, and it is present in other blood diseases, but has been denied in some cases of pernicious anaemia (Moffitt).

Whatever the cause of the improvement, it is highly probable that the subsequent deterioration is due to other related structures taking over the functions of the spleen ('haemolymph-nodes, accessory spleens, Kupffer cells in the liver, and perhaps ordinary lymph-nodes'), although here, also, no positive evidence has as yet been produced. Assuming that the cause of the disease has not been removed, the logical sequence would be that when these auxiliary organs have sufficiently developed the interrupted course of the disease would be resumed.

As to the few patients who up to the present time have continued well after operation, if the curative action of splenectomy is denied, one must assume either that the causative factor has for reasons unknown ceased to operate,

or that the auxiliary organs have failed to develop into pernicious activity.

INDICATIONS FOR SPLENECTOMY IN PERNICIOUS ANÆMIA.—In what types of pernicious anaemia, then, should splenectomy be undertaken? One of two lines may be followed, and it is as yet too early to say which, if either, is correct. If splenectomy merely induces a remission—and this is at present the opinion of the majority of observers—it should be logical to undertake it only as a last resort, when all other measures have proved unavailing, and only with the hope of prolonging life. Even under such limitations, however, the procedure has already proved its value, and in several cases moribund patients have been brought back to life of comparative well-being for many months. Assuming, on the other hand, that an occasional patient may be, for practical purposes, cured of the disease, and giving due weight to the view that greater and longer continued improvement is obtained if the operation is performed before the disease has reached its final stage, it would then be advisable to undertake it as soon as possible. Another factor that may prove to be decisive is whether or not increased haemolysis can be proved. In those cases with clinically enlarged spleens, icteroid appearance, and increased urobilin output, without increased resistance of the erythrocytes, the prognosis is distinctly more favorable than in the opposite types. The condition of the bone-marrow is also important, splenectomy being contra-indicated if the bone-marrow is persistently aplastic. It has also been a matter of clinical observation that those individuals in whom spinal-cord symptoms have already developed are less apt to be helped by the operation.

SUMMARY OF RESULTS OF SPLENECTOMY IN PERNICIOUS ANÆMIA

1. Of the 153 patients studied, 19.6 per cent. died within six weeks; a distinct improvement in the clinical condition and in the blood picture occurred in 64.7 per cent., and no improvement in 15.7 per cent.
2. The rather high postoperative mortality (practically 20 per cent.) may be due to poor choice of cases in the early series. As a much greater proportion of the more recent cases has survived the operation, the true postoperative mortality is probably much less than 20 per cent.
3. Of the individuals who showed improvement shortly after operation—nearly two-thirds of the total number—a large number have failed to maintain this improvement, or have since died in a relapse or from intercurrent disease.
4. Although a few have continued in good condition during the period of observation (over two years), in no case can it be said that a cure has been effected, and the blood of these individuals continues to show many of the characteristic signs of pernicious anæmia.
5. On account of the improvement that follows splenectomy, it would appear to be not only a justifiable, but in many cases an advisable, procedure; but in no case should a cure be promised or the operation undertaken except under the most favorable conditions.
6. The best results are obtained if the operation is preceded by one or more transfusions, and those patients who relapse after operation may still be greatly helped by transfusion. Whether or not transfusions would have produced equally good results in the absence of splenectomy is a question that cannot at present be decided.
7. The most favorable results may be expected in indi-

viduals who have not passed the fifth decade, in whom the disease has not progressed for more than a year, and who have a relatively good blood picture (that is, an anaemia that is not of too extreme a degree or of the steady, progressive type). Individuals with enlarged spleens have done better than those in whom the spleen was small or of normal size, as have also those suffering from an anaemia characterized by excessive haemolysis.

8. The opposite of these conditions should be considered as unfavorable factors, as should also the existence of spinal-cord symptoms or the presence of an aplastic bone-marrow.

CHOICE OF TIME FOR OPERATION.—On general principles it may be said that splenectomy should be undertaken as soon as the diagnosis is definitely settled. In certain instances, however, exceptions must be made. A "crisis of deglobulization" in haemolytic jaundice or a severe hemorrhage from a mucous membrane in Banti's disease would indicate postponement of the operation until the conditions are more favorable. The existence of a possible contributing factor, as indicated, for example, by the finding of a positive Wassermann reaction or of malarial organisms, would naturally postpone operation until the influence of such factors had been, so far as possible, eliminated. When the anaemia is severe—and this applies particularly to pernicious anaemia—a series of blood transfusions (often four or more) should precede operation. If the patient's blood in this way is temporarily enriched, not only is the operative risk lessened, but more lasting benefit ensues. Good results have been obtained in pernicious anaemia by the subcutaneous or intraperitoneal injection of splenic extract,⁸⁷ and in cases where the so-

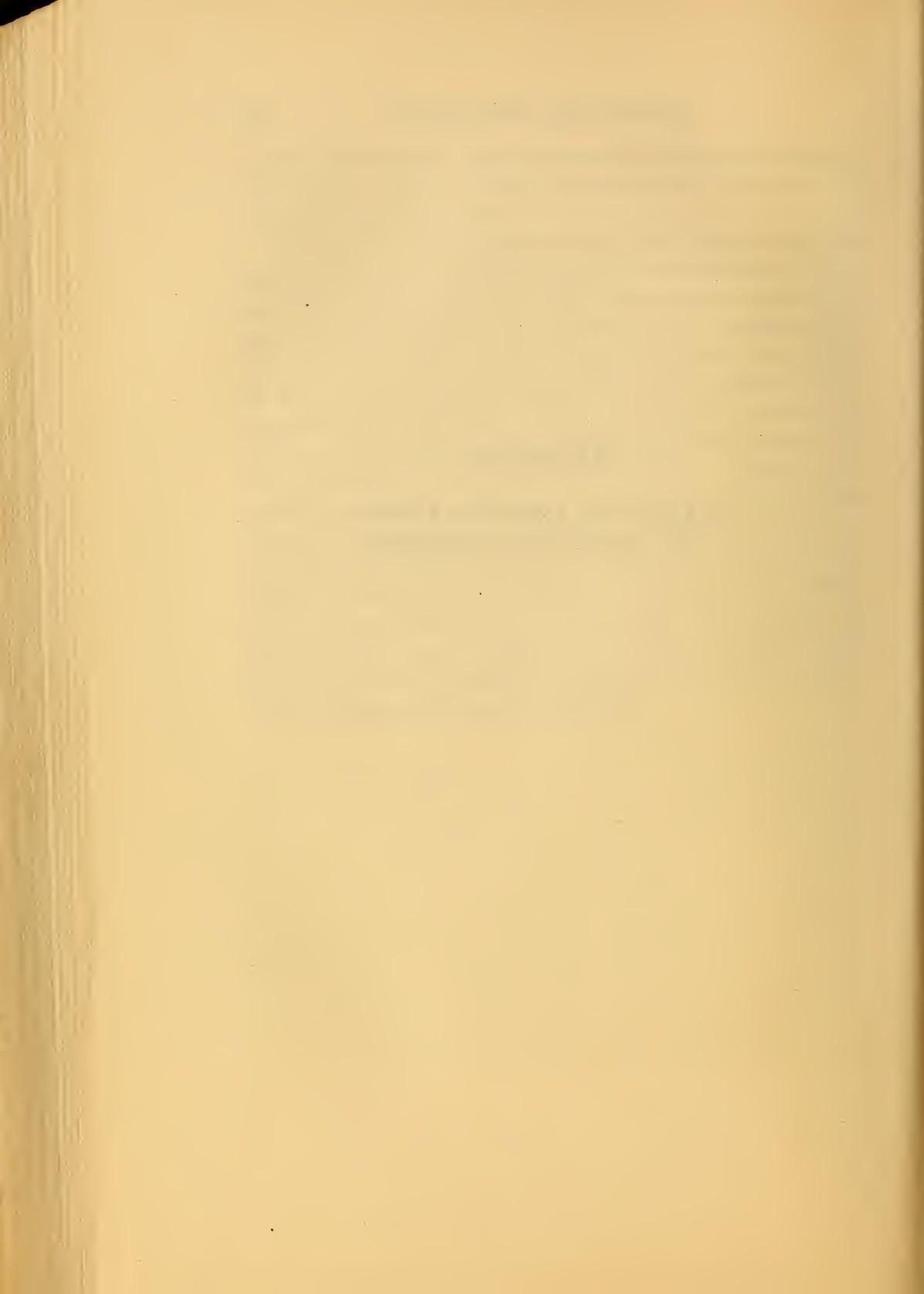
called " blood crisis " fails to materialize after splenectomy, indicating a failure of the bone-marrow to respond, this procedure might be tried.

It may be said, in summarizing, that splenectomy has already proved its worth in many clinical conditions. Even in those conditions in which the early enlargement of the spleen is apparently of a protective nature, evidence exists to show that after a certain stage its hyperplasia results in an increased functioning that is more harmful to the organism than otherwise. When this stage is reached, removal of the spleen usually effects at least a temporary improvement in the patient's condition. Splenectomy should certainly be given careful consideration in all the diseases characterized by evidences of increased blood destruction, and the fact must be emphasized that in early Banti's disease and the haemolytic jaundices the results have been excellent and in many cases have practically amounted to a cure. Such an operation, however, should be regarded conservatively, so long as so much of the physiology of the spleen remains unknown.

PART III

SURGICAL OBSERVATIONS

BY CHARLES H. FRAZIER



CHAPTER XV

THE SURGICAL TREATMENT OF LESIONS OF THE SPLEEN

IN the first century Pliny made the observation, of merely historical interest to us, that sometimes the spleen is a peculiar hindrance to runners, so that it is burned away from those who are incommoded by it; also it has been said that the Romans removed the spleen from some of their gladiators to give them greater endurance in contests. It is, however, a far cry from that day to the nineteenth century, and it was only toward 1860, in the latter part of this century, that splenectomy was admitted to the roll of accepted and approved surgical procedures, while the most important contributions to the surgery of the spleen have been offered during the past five years.

The lesions for the treatment of which the surgeon is called upon to operate are varied and numerous. Beginning with injuries, such as wounds and rupture, there were added wandering spleen, malarial spleen, abscess of the spleen, Banti's disease, the two types of haemolytic icterus, and, finally, pernicious anaemia.

Gunshot and Stab Wounds.—The spleen enjoys a measure of protection from its position beneath the left costal arch, but occasionally it is injured by bullet and stab wounds. As with all injuries to the spleen, hemorrhage is at once profuse and alarming and, unless surgical relief is immediately available, the patient dies of shock or collapse. Should the splenic artery itself or the vein be punctured with or without penetration of the spleen, hemor-

rhage will be so profuse that the life is lost before surgical treatment can be applied. The diagnosis can be only conjectural, but suspicion should be aroused by the constitutional signs of profuse hemorrhage, with perhaps dul-

TABLE LXXI
RESULTS OF THE TREATMENT OF 52 CASES OF STAB WOUND BY VARIOUS METHODS

Operation	Total cases	Recovered	Died
Splenectomy.....	14	9	5
Suture.....	24	22	2
Tamponage.....	12	10	2
Treatment not specified.	2	1	1
Totals.....	52	42	10 19.49 per cent.

TABLE LXXII
RESULTS OF THE TREATMENT OF 82 CASES OF GUNSHOT WOUND BY VARIOUS METHODS

Operation	Total cases	Recovered	Died
Splenectomy.....	40	25	15
Tamponage.....	18	10	8
Suture.....	9	5	4
Partial excision.....	1	1	—
Cauterization.....	1	—	1
Treatment not specified.	6	1	5
Death previous to opera- tion.....	1	—	1
Wounds not detected at the operation.....	6	—	6
Totals.....	82	42	40 48.2 per cent.

ness on percussion in the left flank or when the site or direction of the wound points to the spleen. Immediate operation is imperative, and the treatment of the injured organ will depend somewhat upon the extent and character of the wound. Bullet and stab wounds may be effectively dealt with by suture, but when there is more or less ex-

tensive laceration, when hemorrhage cannot be otherwise controlled, or when the vessels of the pedicle are involved, splenectomy is clearly indicated. In superficial wounds the insertion of a free epiploon has been used advantageously for the control of hemorrhage. Of ninety-nine cases of wounds of the spleen, 50 per cent. of the sixty-one cases of gunshot wounds and 80 per cent. of the thirty-eight stab wounds recovered (Finkelstein¹¹⁶).

Rupture of the Spleen.—Rupture may be designated as traumatic or spontaneous—the former the result of trauma upon a healthy organ, the latter implying an organ already the seat of a pathologic lesion, often of an inflammatory nature, be it malarial, typhoidal, or tuberculous. Rupture of the spleen follows accidents not unlike those which would cause a rupture of the kidney, but in the majority of instances the kidney will rupture and the spleen escape. The passage over the body of wagon wheels, crushes beneath heavy objects, automobile and railroad accidents, the kick of horse or man, falling upon angular objects, or falls from a distance may be numbered among the possible causes, and together with the ruptured spleen one often finds fracture of one or more ribs.

As with gunshot or stab wounds, rupture of the spleen can be surmised only by the character of the injury, by the evidence of an overwhelming hemorrhage with signs of profound shock or collapse, by the presence of dulness in the flank, accompanied in the occasional case by fracture of the ribs in the left side. While there are instances of spontaneous recovery, the majority of cases of rupture of the spleen would be rapidly fatal without surgical interference. Immediate operation should be the rule whenever rupture of the spleen is suspected, and the measure to be adopted will depend upon the extent and depth of the

wound. If the latter be superficial, haemostasis may be effected by simple suture or, as Kirschner²¹⁴ suggested, by the envelopment of the organ with fascia. At all events, one should give preference to conservative practice, if not thereby adding to the risk of operation. There may be many instances, particularly after massive hemorrhages, when the more radical operation, splenectomy, may be done more expeditiously than one or the other of the conservative methods and should therefore be the method of choice.

MORTALITY OF RUPTURE OF THE SPLEEN

TABLE LXXIII

I. MICHELSSON

Operation	Cases	Recovered	Died
Splenectomy.....	254	166	88
Tamponage.....	24	19	5
Suture.....	10	8	2
Other methods.....	10	6	4
Totals.....	298	199	99 33.2 per cent.

TABLE LXXIV

II. LITERATURE OF THE LAST FIVE YEARS

Operation	Cases	Recovered	Died
Splenectomy.....	68	55	13
Tamponage.....	11	8	3
Suture.....	4	4	—
Totals.....	83	67	16 19.3 per cent.

Of 298 cases in Michelsson's²⁹⁰ collection, treated in various ways (Table LXXIII), the mortality was 33.2 per cent., while, excluding all but those operated upon from 1910 to 1915, the mortality has fallen to 19.3 per cent., or almost one-half (Table LXXIV.)

The prognosis of spontaneous rupture of the spleen is influenced by the nature of the pathologic process. If, prior to the rupture, the patient's condition have been depreciated by a more or less virulent and prolonged infection, as of malaria or typhoid fever, the likelihood of survival after an overwhelming hemorrhage is naturally less than when rupture occurs in an otherwise healthy subject or organ.

Abscess.—Because of its vascularity and the slowness of its blood current the spleen is frequently the site of infection, either by the microorganisms themselves or by infectious emboli. Thus we have the secondary infection of the spleen in the acute fevers, such as typhoid and typhus, or in septicaemia, and the primary involvement of malaria, tuberculosis, syphilis, and echinococcus disease. An abscess of the spleen may develop secondarily to any focus of infection and occasionally may result from a contiguous infection, as from perforation of the stomach or subphrenic abscess. Because of the greater frequency of malaria, dysentery, and typhoid fever, in tropical countries, and of the peculiar susceptibility of the spleen to these infections; abscess of the spleen is of greater incidence in the warm and tropical climates than in the temperate zone.

The diagnosis of splenic abscess is frequently not made until the infection has extended beyond the capsular limits. A palpable spleen or one enlarged and tender may be of little significance and the symptoms may vary according to whether the abscess be located in the upper or lower pole. If in the lower pole, an enlarged and painful swelling may be felt; if in the upper pole, the most suggestive signs are those of diaphragmatic invasion or diaphragmatic pleurisy. While there may be theoretical objections to exploratory

aspiration, it seems to have been practiced frequently without untoward effects and must be recognized as the most reliable of all the diagnostic guides. The diagnosis once established, recourse should be had to surgical intervention, since spontaneous rupture of the abscess usually terminates fatally.

The surgical treatment of abscess of the spleen may imply either a splenotomy or a splenectomy. While the choice of operation must be left to the judgment of the operator, generally speaking the safer operation of the two is splenotomy, and this is especially the case if the spleen be surrounded with adhesions. Splenectomy should be reserved for those exceptional cases where the spleen is free from adhesions and can be removed without the dangers of contamination of the peritoneum. The approach to the abscess in splenotomy may be transpleural, abdominal, or retroperitoneal. The transpleural route is peculiarly adapted to upper-pole involvement, especially when the pole is surrounded by adhesions or the infection has extended to the subdiaphragmatic space or the pleural cavity. The problem thus becomes similar to that of dealing with a left-sided sub- or epi-diaphragmatic abscess. If the visceral or parietal pleura be not adherent, the operation is divided into two stages, being content at the first with suture of the parietal pleura to the diaphragm. Otherwise, after resection of a portion of the ninth, tenth, or eleventh rib in the post-axillary line, the operator proceeds at once to open the abscess through the diaphragm.

To expose and drain an abscess in its lower pole or, if need be, to remove the spleen, ready access is obtained by the abdominal route. The incision runs parallel with the last rib, and the latter should be resected, if necessary

to establish direct drainage. During the exploratory steps and during evacuation of the abscess the peritoneal cavity must be protected from soiling. The retro-peritoneal route to the spleen seems at once the least appropriate and the most complicated. As described by Propping, who speaks favorably of it, the exposure is made through an incision from the tip of the twelfth rib along the lower margin of the eleventh rib. If need be, the twelfth and part of the eleventh rib may be resected. With the finger as a guide, the spleen or the abscess may be reached by following from below upward the surface of the diaphragm.

The immediate operative mortality of abscess of the spleen varies from about 10 to 25 per cent., being lowest for typhoidal abscesses, where the infection at the time of the operation is often attenuated. The ultimate prognosis must be presented in a less favorable light when the splenic abscess is but one of a number of pathological lesions or is merely an intercurrent infection in the course of a prolonged or essentially chronic infection. The early recognition of the lesion and its proper management would be attended with a relatively low mortality were it not for these extraneous and coincident complications.

The Malarial Spleen.—The removal of the spleen from malarial subjects is not necessarily a curative procedure, and the course of the disease, even to a fatal issue, may be uninfluenced. On the other hand, it is also true that in many cases removal of the spleen is followed by a general amelioration of the patient's condition and even by recovery. The indications for splenectomy in malarial subjects are threefold: a chronic malarial fever, a wandering spleen, and a weighty spleen, but, as the mortality is high, the

operation should be reserved for those cases in which invalidism is pronounced and where there are no serious changes in liver or kidneys. Not only does improvement follow because of the elimination of an infective focus, but the removal of a weighty organ relieves the patient of discomfort, restores to normal the relation of the abdominal organs, and removes the hindrance to their circulation. Whether splenectomy should be encouraged in the later stages of malaria with ascites, a clinical state not unlike the third stage of Banti's disease, is open to question. Finkelstein,¹¹⁶ who has had an unusual experience in a malarial climate, considers an operation advisable when the haemoglobin is not less than thirty or forty per cent., when the red blood-corpuscles are not less than 2,000,000, when there is no œdema of the lower limbs, when there is no parenchymatous lesion of the kidneys, when the patient is able to move about. Unfortunately, there are no means of determining serious degeneration of the liver, since the presence of a serious hepatic or renal lesion spells disaster after an operation. In the surviving patients the ascites disappears, and in two of Finkelstein's cases the fluid did not reaccumulate and the condition of the patient left nothing to be desired. The operative problems in malaria are virtually those of any disease in which there may be a perisplenitis and consequent adhesions.

Tuberculous Spleen.—Tuberculosis of the spleen is secondary to a primary lesion elsewhere. Up to 1909, Fischer¹¹⁸ had been able to collect but twelve cases, and since that time there have been but few additions. The operation has usually been performed because of an enlarged and wandering spleen and the presence of tuberculosis, suspected and not discovered until examination of the specimen upon its removal.

Syphilis of the Spleen.—Splenectomy has been performed for both the gummatous and non-gummatous splenomegalies. Giffin¹⁴⁴ speaks of the advantageous removal of the spleen in the non-gummatous splenomegaly associated with syphilis. The spleen has been found to contain spirochetæ, and after its removal there has been a rapid diminution in the size of a previously enlarged liver. Furthermore, whereas before the operation the Wassermann reaction continued positive despite appropriate treatment, after removal the reactions were returned as negative. This relationship of the spleen to the continuation of syphilis, the spleen serving as a favorable medium for the propagation of the organism, is a recent observation and is pregnant with possibilities.

Wandering Spleen.—The spleen, for various causes, may make wider excursions in the abdominal cavity than any other organ. It has been found in the sac of an inguinal hernia and often in the pelvis, where it has been mistaken for uterine or ovarian tumor. It may change its position, as the patient moves from side to side, when it usually gravitates to the lowest point; it may, however, float upwards on the intestines; it may become fixed by adhesion in an abnormal position, a condition to which is applied the term “dislocated spleen.”

In most instances the causes of wandering spleen are acquired, although, in exceptional instances, congenital elongation of the mesentery may permit of a wide excursion. The more common causes are accessions in weight, elongation of the pedicle and ligaments, and relaxation of the abdominal walls often associated with visceroptosis (Glénard’s disease). Consequent upon this wider range of motion, which as a matter of fact occurs

most often in the malarial spleen, certain complications may develop, chief among which are engorgement and twists of the pedicle. The former is a gradual process and may add considerably to the weight of the spleen, to the weight of which, under normal circumstances, the blood contributes forty per cent. Twists or rotation of the pedicle will, of course, aggravate the engorgement and may in turn lead to hemorrhages, gangrene, and even peritonitis. The rotation may vary from 180 degrees to 360 degrees, and upon the suddenness and degree of rotation will depend the development of an acute or chronic symptom-complex. Sudden rotation of the splenic pedicle may determine an abdominal catastrophe, mistaken often for intestinal obstruction or peritonitis, marked by tenderness, abdominal rigidity, vomiting, distention, and by the presence of an enlarged, tender, and palpable swelling. In chronic cases there is not only the splenic enlargement, but, by virtue of its weight and displacement, the spleen may disturb the function of other abdominal organs. Epigastric distress, nausea and vomiting may result from dragging upon the stomach, and a train of symptoms from pressure upon the uterus and its adnexa. Not only may the stomach be dragged out of position, but the tail of the pancreas may be elongated, and the uterus displaced or even prolapsed.

Theoretically, the ideal treatment of wandering spleen is splenopexy. This, however, presumes the presence of a normal organ, a condition rarely found. Since in the vast majority of cases wandering of the spleen is primarily due to splenomegaly of one variety or another, often a malarial spleen, splenectomy is the most satisfactory and most logical treatment.

Tumors and Cysts.—The spleen may be the seat of primary or secondary growths: of the primary growths the adenoma, fibroma, and lymphoma have been found, but not on the operating table. The secondary malignant tumors of the spleen are not of practical moment, but a limited number of primary sarcomata have been disclosed at exploratory operations and removed. They take their origin from the capsule or trabecula, the lymphoid structure, and the endothelial cells. One may suspect the presence of a sarcoma of the spleen should there be an enlarged and tender organ, of nodular surface and firm consistency, with palpable notches and a rapid increase in size. Providing metastases are not already detected, the organ should be removed.

Cysts of the spleen may be large or small, of parasitic or non-parasitic origin. The parasitic or hydatid cyst is quite the most common, and its contents display the features characteristic of hydatid cysts elsewhere. It is interesting to note, however, that they usually originate in the upper part of the organ, and, as they increase in size, they may interfere, by pressure upward upon the diaphragm, with the action of both lungs and heart. When feasible, splenectomy is the operation of choice, but when the removal of the organ seems prohibitive from the standpoint of safety, the operator must be content with incision and drainage.

Non-parasitic cysts of the spleen include the dermoid, serous, blood and lymph cysts. The first mentioned is so rare as not to be of practical moment, while the others are often so small as to pass unrecognized. The blood cyst owes its origin to hemorrhage, either parenchymatous or subcapsular, and is often the result of an injury. A history

of trauma followed by the sudden appearance of a splenic enlargement is a significant diagnostic feature. Other diagnostic signs include a tumor of the left hypochondrium of a cystic nature, pain and tenderness, embarrassment of respiration if the cyst be of large dimensions, and, more specifically, a creaking sensation communicated to the examining hand, as with respiration the roughened surface of the cyst and the abdominal wall come in contact.

Splenomegaly.—Of the pathological conditions of the spleen in the treatment of which splenectomy is an accepted method of procedure there remain to be considered the splenomegalies with anaemia. The classification adapted in this monograph recognizes the following types:

1. Gaucher's disease (large-celled splenomegaly).
2. Banti's disease.
3. von Jaksch's disease (*pseudoleukæmia infantum*).
4. Chauffard-Minkowski or congenital form of haemolytic jaundice.
5. Hayem-Widal or acquired form of haemolytic jaundice.
6. Pernicious anaemia.

These several types of splenomegaly have been treated *in extenso* in earlier chapters; suffice it, at this juncture, to speak briefly of their surgical aspects. As a general fundamental principle, the best results may be expected from splenectomy in those diseases in which there is evidence of increased blood destruction. In this category would fall haemolytic jaundice and some cases of pernicious anaemia. Good results have also been obtained in Banti's disease and in the few cases of Gaucher's and von Jaksch's disease that have been studied, but in accepting these as positive indications for splenectomy the proviso must be

made that promising results can be expected in Banti's disease only when the operation is performed in the early stage. The secondary changes which take place in the liver of the patient with Banti's disease are not materially influenced by the removal of the spleen, and when the liver is obviously enlarged the propriety of the operation should be seriously questioned.

In the list of doubtful indications for splenectomy I place pernicious anaemia. The surgery of pernicious anaemia at this writing is *sub judice*. As yet there have been no recoveries following splenectomy, although there have been many instances of prolongation of life, general betterment of the patient's condition, and in lengthening of the periods of remission. The operation, therefore, is not without influence upon the course of the disease and may be practiced in selected cases, with the knowledge, on the part of the patient, that the operation is in the nature of a palliative procedure and not in any sense curative. Furthermore, it must be borne in mind that in some cases repeated transfusions may be almost as effective as splenectomy, and that even when the latter is practiced transfusions may be required.

THE REMOVAL OF THE SPLEEN

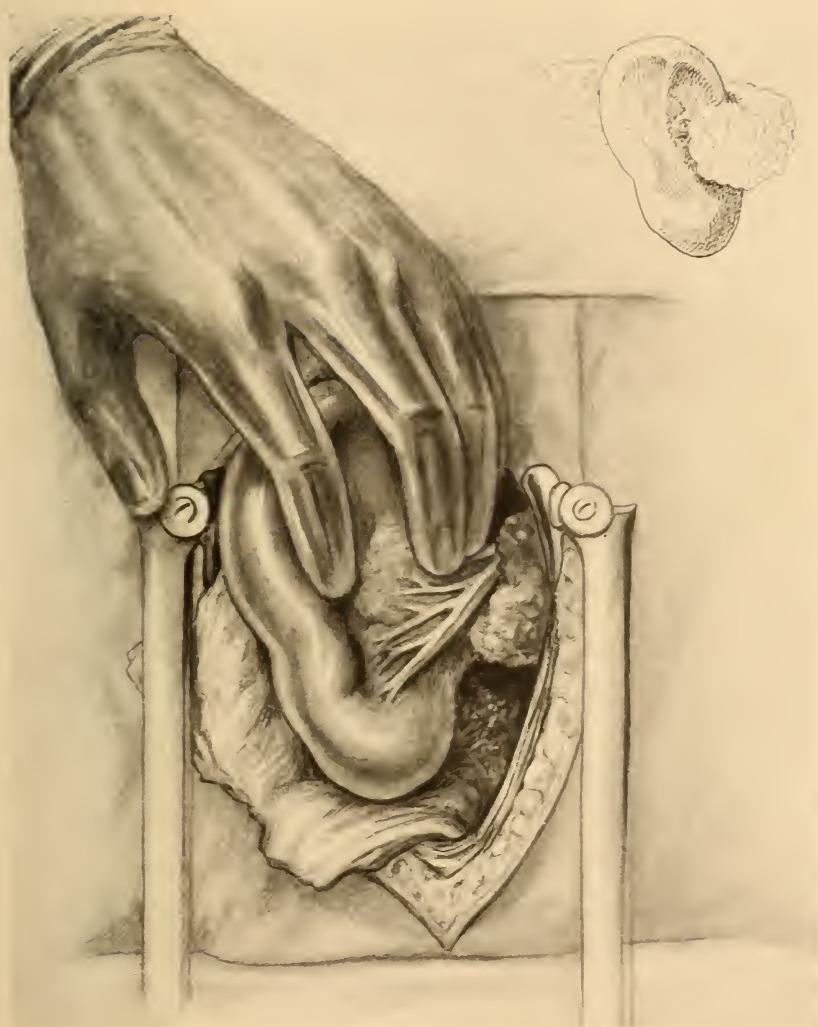
Anatomical Considerations.—The splenic artery, itself a branch of the celiac axis, divides into seven or more branches which supply the spleen, the pancreas, and the stomach. To the latter is given off the *vasa brevia*, passing between the layers of the *gastrosplenic omentum* to be distributed to the great *cul-de-sac*, and the left *gastro-epiploic*, coursing between the layers of the great omentum to the greater curvature. In the operation of splenectomy the splenic artery is exposed for ligation in the pedicle, but

when ligation of the artery is to be substituted for splenectomy, the artery may be exposed, according to Gerster, through an opening in the lesser omentum just above the lesser curvature of the stomach. The first two inches of the artery lie just beneath the posterior parietal peritoneum, and may be brought nearer the level of the anterior abdominal incision by placing an Edebohl cushion beneath the patient's back. The splenic vein, or splenic veins—for there are often more than one in the pedicle—unite with the superior mesenteric to form the portal vein, so that a thrombus of the latter seriously interferes with the splenic circulation. The pedicle of the spleen varies in length. The shorter the pedicle the more difficult are all the stages of splenectomy, including the delivery of the organ and the ligation of its vessels. It is comprised of connective tissue and fat, occasionally of lymphatic nodes, some accessory spleens, and the tail of the pancreas (Plate X), and through the pedicle pass the splenic artery, and one or more veins. The smaller vessels to the stomach lie anterior to the pedicle. In addition to the splenic artery and veins which enter the spleen at the hilum, there is a leash of vessels which enter the spleen through what Richards calls a secondary hilum near the lower pole. These vessels are carried in a fold of peritoneum that is reflected on the surface of the splenic flexure of the colon.

The capsule of the spleen is so firmly adherent to the surface that attempts at separation would be attended with laceration of the pulp; therefore a subcapsular splenectomy is not feasible.

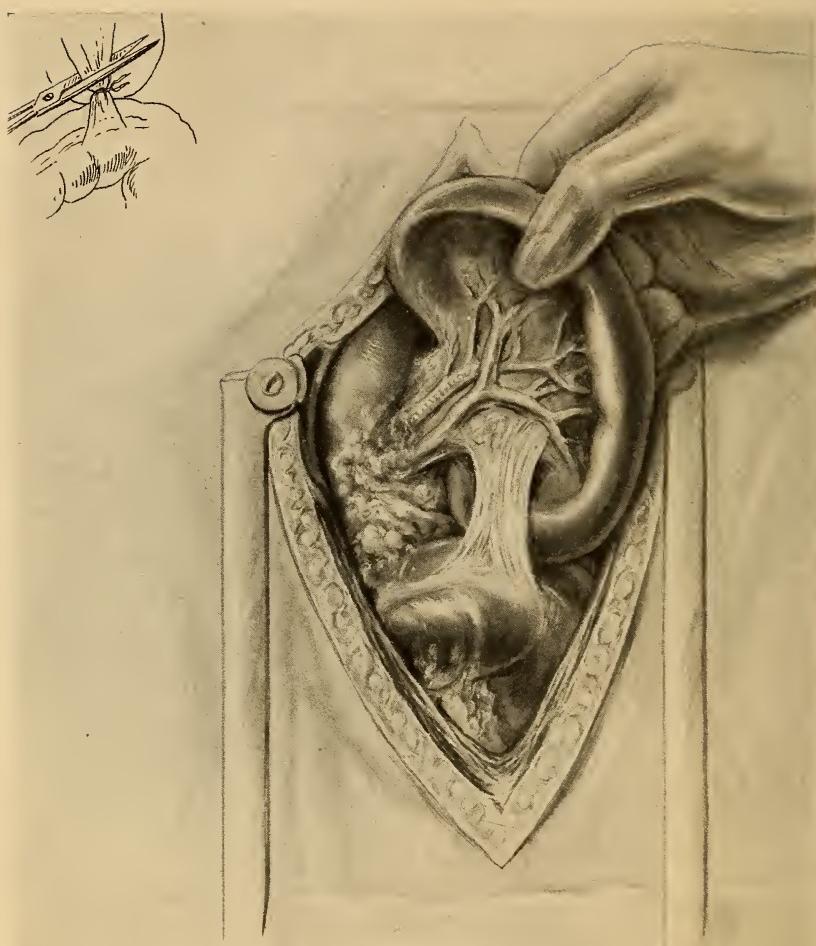
A knowledge of the relationship of the spleen to the contiguous structures is of practical moment. The superior pole is in close relation with the diaphragm and the

PLATE X



The relationship of the tail of the pancreas to the posterior aspect of the pedicle.

PLATE XI



The peritoneal prolongation between the spleen and the splenic flexure of the colon, which must be divided before the organ can be completely mobilized.

lower pole through a peritoneal reflexion with the splenic flexures of the colon. In the pancreatic notch behind the hilum lies the tail of the pancreas, and its relation to the structures of the pedicle has already been mentioned. This is a matter of practical consideration, since, unless care be taken to avoid it, a portion of the tail may be amputated in division of the pedicle after ligation. This accident has occurred more than once. So close does the greater curvature of the stomach lie to the pedicle of the spleen that a portion of the wall has been inadvertently excised, in one instance with a fatal result.

The spleen is maintained in its position under normal conditions by a number of ligaments, by the gastrosplenic ligament, the lienorenal, by an occasional band to the lower pole derived from the phrenocolic ligament (Plate XI), and by its pedicle. Under abnormal conditions—and it is mostly under these that splenectomy must be performed—the ligaments above mentioned are fortified by adhesions of such density and vascularity that they may make the operation, if not impossible, at least surrounded with many difficulties. The most troublesome adhesions are those from the outer surface and upper pole to the diaphragm, but there may be others to the stomach, large bowel, and parietal peritoneum. W. J. Mayo calls attention to the vascular connections in the deeper portions of the gastrosplenic omentum, which pass inward and backward to anastomose with vessels along the spine and crux of the diaphragm. These vascular connections must be divided before the spleen can be delivered.

Blood Transfusion.—Blood transfusion frequently may be called for, either in the preparation for operation or in the after-treatment of the splenectomized patient. Fol-

lowing the alarming hemorrhages of wounds and rupture of the spleen, the transfusion of blood, if in the emergency a suitable donor can be found, may be a life-saving remedy. In such situations the transfusion is performed immediately after the operation. When splenectomy is contemplated in the chronic case, be it Banti's disease, haemolytic icterus, splenic anæmia, or pernicious anæmia, transfusion plays a very important rôle in the preparation of the patient for operation. Should the haemoglobin be below thirty or forty per cent., the patient should be transfused forty-eight hours before the time set for the splenectomy and again after the operation, should the latter be attended with much loss of blood. It is, however, not only with reference to the blood picture that inquiry be made into the patient's condition. A careful, systematic examination of the cardiovascular and renal systems should be made to determine whether, irrespective of the anæmia, the patient be a good operative risk. It may well be in certain cases that a patient with 2,000,000 red blood-corpuscles may be a better hazard than one with 3,000,000 corpuscles, but with other handicaps.

It is in pernicious anæmia, however, that transfusion plays the most important rôle, not only in the preparation of the patient for the operation—the phase of the subject with which we are most concerned—but also in preventing relapses and prolonging the period of remission. Parenthetically it may be said that if no improvement follow transfusion little should be expected to follow a splenectomy. The effects of one may be said to forecast the effects of the other. Percy, whose large experience compels a hearing, has evolved a systematic method of managing the transfusion problem to which I attribute, in part at least,

his low mortality. He has adopted what he styles the step-ladder method of preparing his patients by transfusion; he prefers whole blood to defibrinated blood or blood treated with sodium citrate; the average number of transfusions for each patient varies from three to five, the average amount of blood is 640 c.c., and the average interval between the time of the first transfusion and the splenectomy is twenty days. In almost every case improvement follows immediately after the first transfusion, and continues by "step-ladder" progression with successive transfusions until the patient is in a state prepared for operation. At the conclusion of the operation, and while still on the table, the patient is transfused again. According to Minot, the most auspicious time for the splenectomy is from four to ten days after the transfusion, when the Howell-Jolly bodies appear in greatest number, together with a rise in the leucocyte count and an increase in the blood-platelets and reticulated cells.

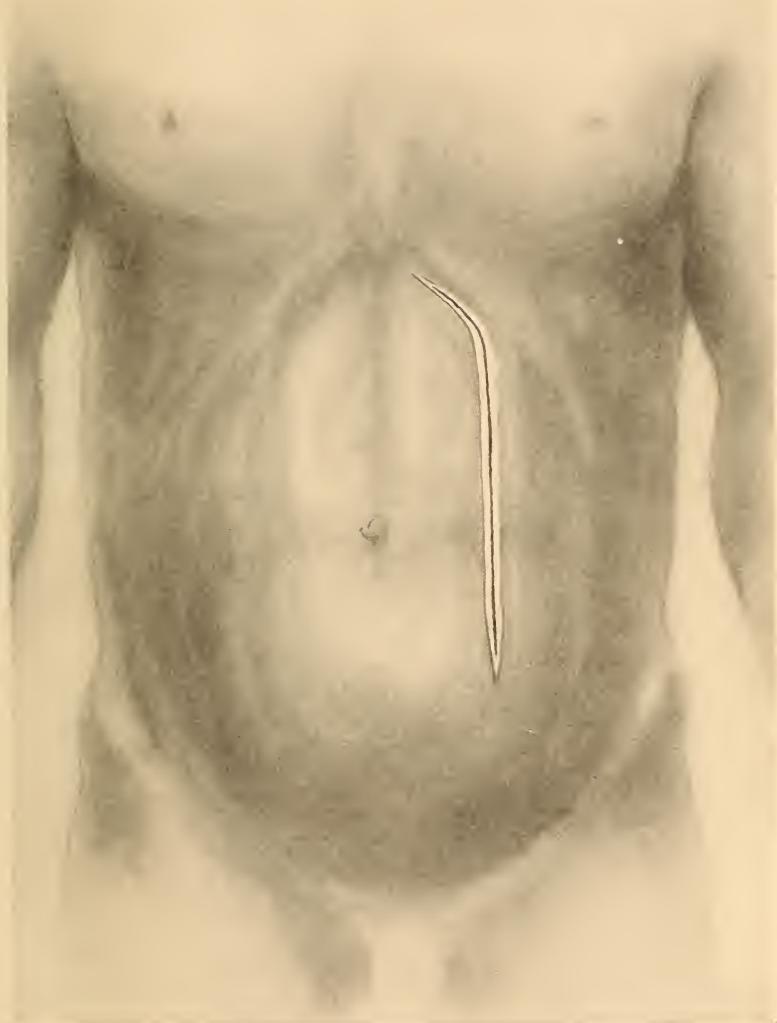
The effect of transfusion upon the subject of pernicious anaemia varies somewhat with the stage of the disease. In the early cases transfusion may give a remission of several months and seem almost as beneficial and enduring in its effects as splenectomy. In the late cases the effect is only transitory, a matter, perhaps, of two or three weeks. But, while the transitory effect is fully recognized, transfusion should not be discarded, since it is in itself a humane measure, giving to the patient a sense of *bon faisance*, increasing the appetite and general bodily comfort, and unquestionably prolonging life.

The obligation of the surgeon in the selection of a suitable donor need only be mentioned. That vigilance is required to protect the patient from transmissible diseases

may be inferred from the transmission of syphilis in one reported case where the donor, denying exposure to infection, had the primary lesion at the time of the transfusion. In the selection of a donor the matter of blood compatibility must be investigated with great care in order to preclude even the remotest possibility of haemolysis or agglutination.

Technic of Splenectomy.—The removal of the spleen under certain conditions is an operation devoid of any peculiar difficulties. When the spleen is not enlarged, or when the adhesions are few and so readily divided that delivery of the organ is a simple manœuvre, splenectomy might be compared with an uncomplicated nephrectomy. The problems are somewhat similar: the manner of approach (that is, the incision), the isolation of the organ and its delivery, the management of the pedicle, and the after-care of the wound. But the difficulties of the difficult splenectomy quite exceed the difficulties of the difficult nephrectomy, and the chief point of difference between the two lies in the presence of dense vascular adhesions with which the spleen may be surrounded. For the preferable line of incision one must choose a midline incision, between an incision at the junction of the middle or outer third of the rectus and an incision in the linea semilunaris. Each of these incisions may have its advantages under certain conditions, or perhaps no one should be used to the exclusion of others. Personally, I prefer a left-sided Bevan incision, in the outer rather than the inner half of the rectus (Plate XII), as advocated by Balfour.²⁷ The upper limb of this incision is projected across the rectus an inch below the costal margin, and the flap thus formed, when grasped with a pair of forceps, may serve as a retractor.

PLATE XII



Left-sided Bevan incision for splenectomy.

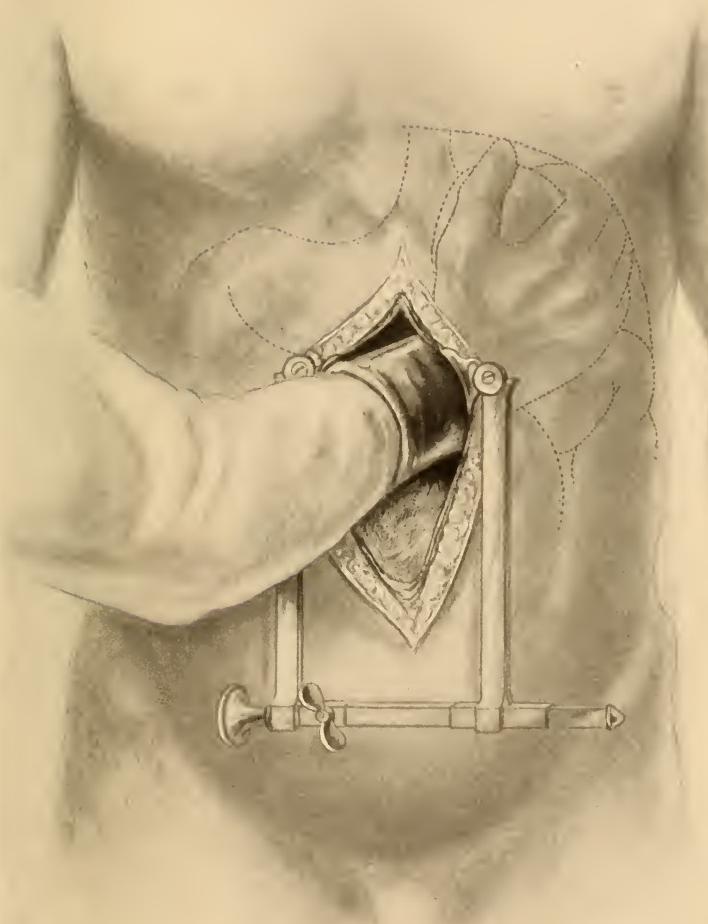
Owen Richards³⁷² and Aly Bey, whose experience has been chiefly with large and adherent spleens of the Egyptian splenomegaly, prefer a vertical incision, stretching half-way between the costal angle, the lower margin of the thorax, about two or three inches from the median line, and extending downward a distance of six or eight inches. If the incision be in the midline, access to the outer surface of the spleen is not adequate, and if more external than the incision above described, the costal margin prevents the prolongation of the incision high enough to give access to the vault of the diaphragm. With this left rectus incision Richards has but once had to add a transverse cut in the rectus to give additional room for manipulation.

Percy³⁴⁴ has quite recently adopted a midline incision and claims for it advantages over those nearer the outer margin of the rectus. No doubt he has been influenced in his selection of a midline incision by his practice of thoroughly exploring the abdomen, particularly the region of the appendix and gall-bladder, in search for an infective focus as having some bearing upon the pathogenesis of pernicious anaemia. However this may be, he has found an associated lesion of the gall-bladder or appendix in many of his cases. In a series of twenty-four splenectomies he had performed twenty-one combined operations, seventeen splenectomies, appendectomies, and cholecystectomies; three splenectomies and appendectomies, and one splenectomy with the removal of carious teeth.

After exploring the abdomen with particular attention to the condition of the gall-bladder, biliary passages, and the liver itself, the operator proceeds systematically to prepare the spleen for delivery, directing his attention first to freeing its upper pole and outer surface. To accom-

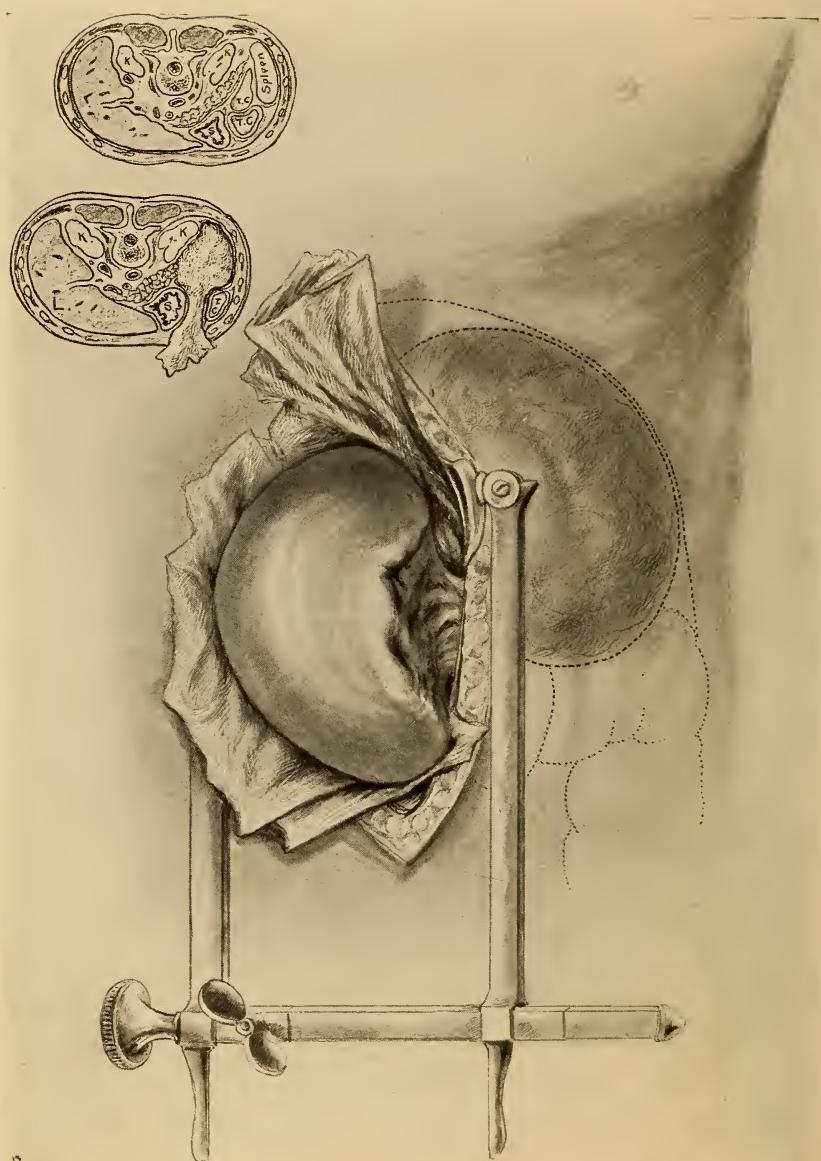
plish this, the right hand is inserted in the left subdiaphragmatic space and by blunt dissection with the fingers the adhesions are separated as close to the spleen as possible (**Plate XIII**). This step of the operation is perhaps the most difficult, because the field is not within view and hemorrhage may be profuse. It may be necessary, should the adhesions be dense, to double-clamp and divide them with scissors. At this stage hemorrhage may be temporarily controlled by the use of a hot pack (**Plate XIV**), postponing until later ligation of those points still bleeding after the spleen has been removed. The hot pack, a feature of W. J. Mayo's technique, serves a two-fold purpose: permanently controlling a number of the bleeding points, especially those of venous origin, and, secondly, serving as a support for the subsequent manipulation and mobilization of the spleen. After the adhesions to the upper and outer surfaces are free and the space tamponed, the lower pole is drawn up and the lienorenal ligament with its vessels divided between two ligatures. Before the pedicle can be satisfactorily dealt with there remains to be divided the gastrosplenic omentum, between the layers of which pass the *vasa brevia* on their way to the stomach. The close relationship of the stomach to the spleen must be borne in mind at this juncture, since in the division and ligation of the gastrosplenic ligament the stomach may be inadvertently opened. Freed from all attachments save the pedicle, the spleen is now mobilized cautiously until the pedicle is exposed. To accomplish this an assistant supports the lower pole, and the operator, with his right hand upon the upper pole and his left depressing the outer margin of the abdominal incision, by gentle traction and pressure manœuvres the spleen through the abdominal wound. The

PLATE XIII



First step in the mobilization of the spleen. With the right hand the operator separates the adhesions between the superior surface of the spleen and the diaphragm.

PLATE XIV



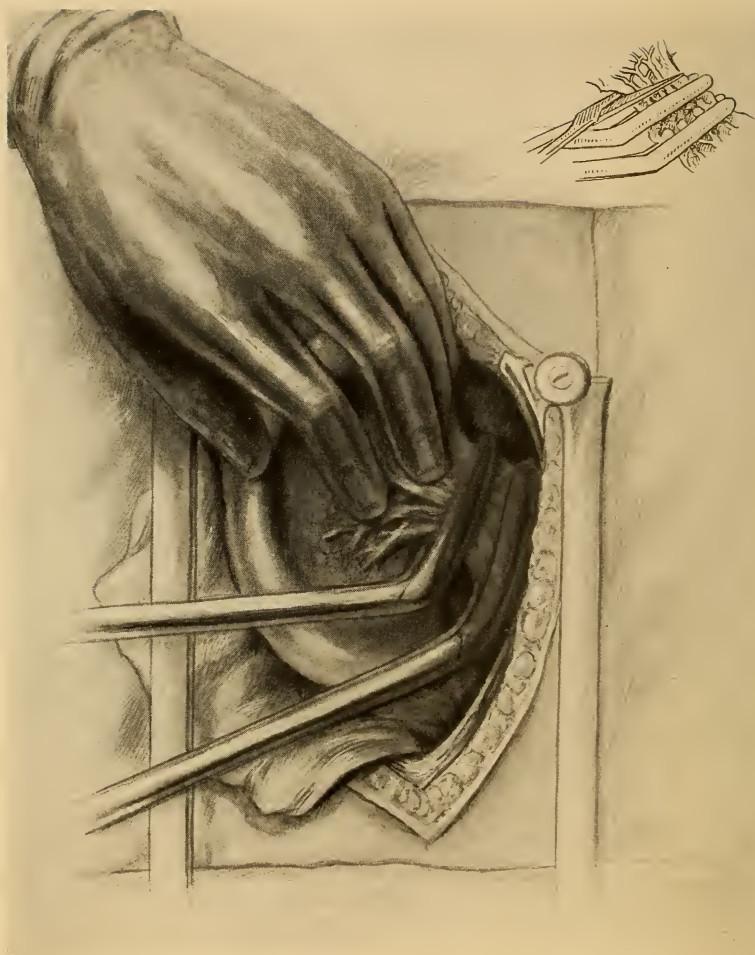
To control bleeding after mobilization of the spleen a large tampon of gauze, wrung out in hot water, is introduced into the left hypochondrium.

PLATE XV



Tractional ligation after isolation of the vessels of the pedicle by blunt removal of fat and connective tissue.

PLATE XVI



The double-clamp method of dealing with the pedicle.

difficulty in delivery has been said to be in direct proportion to the firmness of the adhesions and sometimes in inverse proportion to the size of the spleen. When the spleen is large and weighty sufficient support must be applied to prevent undue traction and laceration of the structures of the pedicle.

The most important, though not always the most difficult, step of splenectomy is the treatment of the pedicle. Grave hemorrhage may attend the process of ligation should the ligature slip and the vessel retract, or, after the patient has been returned to bed, should an insecure ligature become dislodged. As a general principle, it is a safer plan to ligate the pedicle in sections as the vessels present themselves (Plate XV), although when the pedicle is short it may be necessary to apply clamps and ligate *en masse*. By blunt dissection of the peritoneal covering and connective tissue each vessel is isolated for an inch or more and divided between two ligatures, the distal ligature being tied close to the spleen. The ligatures should be tied as far apart as possible and the vessel divided near the spleen so as to leave a long stump centralward. There are usually two or more veins to be dealt with and one or more arteries, according to whether the artery divide before it enters the hilum. Theoretically the artery should be tied first in order that the spleen may at least partially empty itself of its blood content.

Fractional ligation of the pedicle must be discarded for ligation *en masse* when the pedicle is too short. Very much as in nephrectomies, the clamp is used when the pedicle is so short that the vessels cannot be easily exposed. Two curved rubber-covered forceps are applied to the pedicle (Plate XVI), three-quarters of an inch apart, and the

spleen cut away without regard to back bleeding (Mayo), but after the splenic artery has been tied or clamped bleeding from the spleen itself is no great loss to the patient, although, as graphically described by Richards, this back bleeding is "mussy and demoralizing." A catgut ligature is thrown about the pedicle as the proximal clamp is loosened and the ligature tied in the compressed area. The distal pair of forceps is used to steady the pedicle while the proximal ligature is tied, and for further security a second ligature is employed. In the application of the clamps by this method the operator is again cautioned not to include the wall of the stomach or the tail of the pancreas. After the pedicle has been ligated and the spleen removed the proximal stump should be given a final inspection to make sure of the security of the ligature. If there still remain oozing points, these should be taken care of, and should the stump of the pedicle be broad it may be overrun with a fine, continuous catgut suture or covered with omentum.

Gerster believes that ligation of the arterial supply at points more accessible than the deeply-situated splenic pedicle will greatly facilitate matters in difficult operations. These points are the splenic artery close to the celiac axis and the left gastro-epiploic, where it reaches the stomach wall. The celiac axis is exposed and the splenic artery ligated through an opening in the lesser omentum; the left gastro-epiploic is ligated just before it begins to send off branches to the arteries and posterior surfaces. With the arterial streams under control, Gerster feels a greater sense of security in dealing with the vessels of the pedicle should difficulty be encountered or accidents happen. After the vessels of the pedicle have been dealt with,

the preliminary ligatures of the splenic and left gastro-epiploic artery, if only temporary, may be released, so that if any arterial branches in the pedicle have been overlooked they may now be tied. There is no serious objection, according to Gerster, to allowing the ligatures to remain. While the pancreatic and vasa brevia branches of the splenic artery are shut off from their direct arterial source, the pancreas and stomach wall still have an abundant arterial supply through other branches and anastomosing relationships. This suggestion of temporary distant ligation of the vessels may prove of service in exceptional cases, but so far as I know it has not as yet been put into actual practice.

The pedicle divided and the spleen removed, the hot pack is slowly withdrawn and an inspection made for points of hemorrhage from divided adhesions. These are best controlled with mounted ligatures. With haemostasis satisfactorily established, the operation is concluded by closure of the splenic space. This Mayo regards as exceedingly important and introduces what he terms a snaking suture, as follows:

"With catgut on a small curved needle, the raw space beginning at the tied splenic vessels is closed as well as possible. The margin of the lienorenal ligament, on the outer side, is sufficiently firm to hold a suture, but on the inner side such bits of tissue must be caught here and there as can be done safely until the bleeding vessels are compressed. The last sutures come well down in the dia-phragm and had best be applied during cardiac diastole and during expiration."

The purposes of the operation fulfilled and the field dry, the wound is closed without drainage, although to

this general rule exceptions may be taken—on the one hand if the wound be not entirely dry, or if the field may have been soiled by an accidental injury to stomach or colon.

For many reasons the most serious complication of splenectomy is hemorrhage. The fragility of the splenic veins exposes them to laceration when too much traction is put upon the pedicle; in the case of an atheromatous splenic artery a ligature may tear through; should a ligature or clamp slip from the pedicle the stump of the divided vessel quickly retracts beneath the costal margin; in the separation of adhesions the capsule may be torn and the splenic pulp bleed freely; the adhesions themselves may be extremely vascular and contain a well-developed artery and a number of varicose veins; ligation of the *vasa brevia* or the *gastrosplenic omentum* may be troublesome. The spleen itself has been described as an elastic bag full of blood under pressure, and the splenic vessels, when cut, bleed furiously from either end. Its blood content equals forty per cent. of its weight, and this in enlarged spleens may amount to more than a quart. It matters little, however, whether the artery or vein be first ligated: the amount of blood it contains after removal is practically the same in either case.

While the sources of hemorrhage are many, the impression must not be gained that a splenectomy is usually attended with the loss of a considerable quantity of blood. In the absence of adhesions I have completed the operation with the loss of not more than two teaspoonfuls of blood, and, though the potential sources of hemorrhage are numerous, with careful attention to methods of prevention and to methods of control, even in difficult cases, the

loss of blood may be within reasonable limits. I have had no experience with the preoperative administration of adrenalin, which is said to cause marked shrinkage of the organs, but have been content with the effect of normal saline solution, intravenously or with blood transfusion, when the amount of blood lost at the operation might retard the patient's convalescence. In cases of pernicious anaemia Percy³⁴⁴ routinely transfuses the patient while still on the operating table.

As complications of splenectomy, apart from hemorrhage, should be mentioned injuries to adjacent viscera. Mention has been made of the proximity of the stomach and of its occasional injury during splenectomies and of the intimate relationship of the tail of the pancreas to the pedicle and hilum. Even when a portion of the tail of the pancreas has been included in the ligature of the pedicle, no serious consequences, such as fat necrosis from the pancreatic secretion, have been reported.

The operative sequelæ of splenectomy are varied. With the exception of shock and collapse, as in cases of grave hemorrhage, the most frequent complication is bronchitis or pneumonia. The susceptibility of the splenectomized patient to infection is a factor to be reckoned with, but this alone does not explain why the lung should be the site of election for the coincident infection. Nor can the relative frequency of pneumonia be attributed to a transitory paralysis of the diaphragm, because these pneumonias develop on the right side as well as the left. Whatever may be the cause, the fact remains that pneumonia is a common complication; and in one of my cases, a splenectomy for Banti's disease, the patient developed after the pneumonia a pneumococcic peritonitis, from both of which, however, she

made an excellent recovery. Of the causes of death other than those attributable to the anæmia, I may mention shock, pain, hemorrhage, pneumonia, suppression of urine, mesenteric thrombosis, portal thrombosis, and acute dilatation of the stomach.

The operative risks of splenectomy must of necessity vary widely; the condition of the patient at the time of operation, the disease from which the patient is suffering, and, more particularly, the stage of the disease at which the operation is performed, the care and judgment exercised in the selection of cases, not to mention the skill and experience of the operator—these and other factors affect the mortality. As our knowledge of the indications for operation has developed, as our judgment in the selection of cases has grown riper, as the advantages of transfusion have been made use of in the preparation of our patients and their after-care, as the pitfalls are recognized and the means of avoidance understood, the mortality of splenectomy has been very materially reduced.

The largest number of splenectomies by a single operator was the series of Finkelstein,¹¹⁶ who recorded his mortality for 66 cases from 1903 to 1913 as 38 per cent. Carstens collected a series of 739 splenectomies with a mortality of 27.4 per cent. In a series of 75 splenectomies in malarial subjects, representing six operators, the death-rate was 34.7 per cent. That the risk of this operation can be and has been reduced materially may be inferred from the reports of more recent experiences with pernicious anæmia when the mortality rate has been reduced to 8 per cent. This has been made possible by a clearer understanding of the problems of the operation, by the judicious employment of transfusion, and greater care in the selection of cases.

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